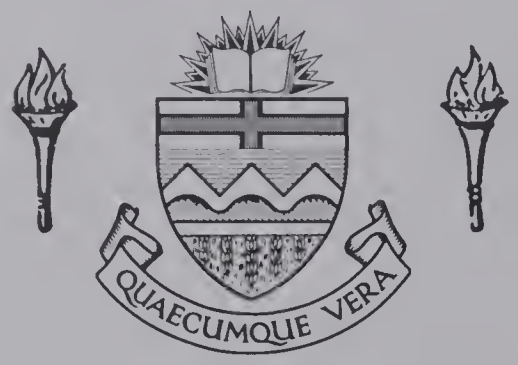

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EFFECTS OF DIETARY COPPER AND PROTEIN ON THE FATTY ACID COMPOSITION OF PORCINE DEPOT FAT

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Effects of dietary copper and protein on the fatty acid composition of porcine depot fat" submitted by James Ingham Elliot, B.S.A., M.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Two experiments were conducted between 1965 and 1968 to study the effects of including 0.1% $CuSO_4 \cdot 5H_2O$, to supply approximately 250 ppm of supplemental copper, in the diet of Hampshire × Yorkshire pigs from weaning to market.

In Experiment 1, 24 barrow pigs were individually fed a barley-fishmeal diet with or without supplemental copper to a scale based on liveweight. Three pigs from each dietary group were slaughtered at 26, 47, 70 and 90 kg, and samples of the outer backfat, inner backfat and perinephric fat were collected. These were analyzed by gas-liquid chromatography to determine their fatty acid composition. The fatty acid composition of the depot fats from non-supplemented and supplemented pigs was compared.

In Experiment 2, 64 gilts and barrows were individually fed barley-fishmeal, barley-meat meal, barley-soybean meal, or barley-rapeseed meal diets, with or without supplemental copper, ad libitum. Samples of the outer backfat were obtained by biopsy at 34, 45, 57, 68 and 79 kg liveweight, and at slaughter (88 kg) samples of the outer backfat, inner backfat and perinephric fat were taken. The fatty acid composition of the depot fats from non-supplemented and supplemented pigs was compared within and between sources of supplemental protein.

In Experiment 2, data on feed intake, average daily gain, feed conversion and carcass measurements were obtained in addition to fatty acid composition. Copper supplementation of the diet did not significantly affect daily gain or feed conversion, however, these parameters were significantly affected by source of supplemental protein and sex.

Copper supplementation of the diet did not affect any of the carcass characteristics.

Copper supplementation of the diet increased the proportion of unsaturated fatty acids and decreased the proportion of saturated fatty acids in the outer backfat, inner backfat and perinephric fat. The increase in unsaturated fatty acids was accounted for by increases in the weight % of palmitoleic (16:1), oleic (18:1) and linoleic (18:2) acids while the decrease in saturated fatty acids resulted from decreases in the weight % of palmitic (16:0) and stearic (18:0) acids present in the depot fats.

The composition of the depot fat of copper supplemented pigs appeared to be affected by the chronological age of the animal at market weight. The older the animal, the more closely the composition of the fat approximated that of the control animals. Age at market was in turn dictated by feeding system, restricted versus ad libitum.

The effect of supplemental copper on depot fat composition was influenced by source of supplemental protein and by sex. Pigs fed barley-fishmeal and barley-meat meal diets supplemented with copper produced a more unsaturated depot fat than did those receiving a barley-soybean meal diet. Copper supplementation of the barley-rapeseed meal did not significantly alter the depot fat composition. Gilts, fed copper, produced a more unsaturated depot fat than did barrows.

Practical implications of the influence of copper supplementation of swine diets on the production of soft fat in pigs are discussed.

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INTRODUCTION

It has been well established that copper is essential for the normal growth, development and functioning of plants and animals. Diets composed of normal feedstuffs seldom contain less than 5 ppm of copper, a level considered adequate in terms of the copper requirements of the pig. Beyond the suckling stage therefore, there is little likelihood of true copper deficiency being encountered. Indeed supplementation of most livestock and human diets is not considered necessary and if practiced may result in toxicity.

The pig is an exception to other animals in that it will tolerate and often respond to high level copper supplementation of the diet. The response, when it occurs, is manifested by improved average daily gain and feed conversion. Copper sulfate is widely used in Europe as a growth promotant however, as yet, its use in North America has not been widely accepted. Recently it has been noted that supplementation of practical swine diets with copper may result in alteration of the composition of the depot fat and sometimes appears to be responsible for the production of carcasses with soft fat.

Since experimental evidence regarding the effects of copper supplementation on depot fat composition was limited, it seemed desirable that more definitive information should be obtained. The experiments reported herein outline the results of a study of depot fat composition when pig diets of varying composition were supplemented with copper.

LITERATURE REVIEW

A. INCLUSION OF COPPER IN PRACTICAL SWINE DIETS

1. Historical

The first report of a beneficial response to the use of supplemental copper sulfate ($CuSO_4 \cdot 5H_2O$) in practical swine diets was that of Evvard, Nelson and Sewell (1928; cited by Hawbaker et al. 1961). Braude (1945) reported that pigs, in a new piggery, which had copper rings placed at floor level around the tubular iron pen structure, literally licked the rings away. Subsequently plates of aluminum, brass, copper, magnesium, nickel and tin were placed in two pens and a newly weaned litter placed in each pen. Only the copper and brass (a copper-zinc alloy) plates were licked and the brass one only occasionally. In the same report, Braude (1945) noted that when three groups of pigs were fed a control diet, the control diet plus 25 mg per day of copper (as $CuSO_4 \cdot 5H_2O$), or the control diet plus 50 mg per day of copper from 14 weeks of age to 36 weeks of age, the pigs fed the supplemental copper gained faster and had an improved feed conversion (F.C.), kg feed/kg gain, compared to the pigs fed the control diet. However, this advantage was evident only during the first eight weeks of the trial. In the overall analysis there were no beneficial effects attributable to copper supplementation of the diet. . In a second experiment this response was not observed even in the early weeks of the trial. Carpenter (1947; cited by Hawbaker et al. 1961) reported that growing pigs fed 45 ppm of supplemental copper gained faster than pigs fed a control ration containing no supplemental copper.

Braude (1948) prepared several salt licks which differed only in the metal powder added to them: cobalt, copper, manganese, nickel, tin or zinc. These licks were weighed, placed in two pens and eight pigs placed in each pen. After six weeks the licks were reweighed. The pigs had not touched the control or the cobalt containing licks, they had consumed only a small amount of the licks containing Mn, Ni, Sn or Zn, however, they had consumed 1718 and 1123 g of the Cu containing lick in pens one and two, respectively. Braude (1948) further noted that the older the pigs the less interest they showed in the licks. The licks had no effect on the liveweight of the pigs.

Mitchell (1953) offered six litters, consisting of 55 pigs, free choice access to two creep diets. The basal diet contained 10 ppm Cu while the second was supplemented with Cu (as $CuSO_4 \cdot 5H_2O$) to a level of 150 ppm. Feed consumption of the two diets was measured from two weeks of age until weaning at eight weeks of age. During this six week period the pigs consumed 54.4 kg of the basal diet and 161.0 kg of the copper supplemented diet. He concluded that the creep diet containing 150 ppm Cu was more palatable. However, a later experiment involving 300 pigs and the same level (150 ppm) of dietary copper supplementation (Barber, Braude and Mitchell, 1955a) failed to confirm this observation.

Since these initial observations were reported, numerous experiments have been carried out in various parts of the world to evaluate copper supplementation of practical swine diets. These will be reviewed in the following sections. The results of these experiments have resulted in the widespread use of copper as a dietary supplement in Great Britain and Europe. Braude (1965) estimated that one-third

of all pigs marketed in Great Britain receive supplemental copper. Results of trials in other parts of the world, notably North America, have tended to indicate that copper supplementation may have deleterious effects. Its use as a feed additive in Canada has not been approved.

2. Effects of copper supplementation of the diet on feed consumption, average daily gain (ADG) and feed conversion (FC)

Throughout this review, copper supplementation refers to the addition of 0.1% $CuSO_4 \cdot 5H_2O$ to diets to supply approximately 250 ppm copper, unless otherwise indicated. Barber et al.(1955c) reported that when diets fed to fattening pigs were supplemented with 2.5% of a mineral mixture "XF" which contained 4% of copper sulfate there was a trend towards a greater rate of gain in comparison with the control group which did not receive the mineral mixture "XF". This level of mineral supplementation supplied approximately 250 ppm supplemental copper in the diet. There was no significant effect of supplemental copper on feed consumption, although feed consumption of copper-supplemented pigs tended to be higher. These effects were noted in the first eight weeks of the trial but were not observed during a subsequent ten-week period. Bowler et al. (1955) reported the results of a cooperative experiment run at eight centres in the United Kingdom involving 182 pigs in which diets with and without supplemental copper were fed. Copper supplementation of the diet significantly increased the overall ADG by 0.036 kg and improved FC by 0.04 kg/kg. Improvement in growth was due to improved feed utilization rather than increased feed consumption. Lucas and Calder (1957a) reported improved ADG and FC during the growing period from 20.0 to 47.3 kg in pigs fed diets

supplemented with copper at a level of 250 ppm, however, between 45.5 and 91.0 kg no significant differences in these two parameters were found. Lucas, Livingstone and McDonald (1961) observed that the overall effect of copper on ADG and FC to market weight is a reflection of its effect during the growing period. Barber et al. (1957) reported that copper supplementation of diets fed to fattening pigs increased feed consumption and ADG significantly with a small non-significant effect on FC. Allen et al.(1961) and Lucas (1964) noted that when pigs were fed to a scale based on liveweight, as is done in Europe, the major response to copper supplementation was prior to 45.5 kg liveweight; however, if pigs were fed ad libitum, as is the practice in North America, responses also occurred between 45.5 and 91.0 kg liveweight. The latter response was related to increased feed intake.

Braude (1965) has published an extensive review of the literature on the effect of copper on ADG and FC. All trials reported in the literature up to mid-1965, in which the performance of growing pigs receiving supplemental copper at a level of 250 ppm was directly compared with that of similar control animals, were summarized. This involved 83 trials and a total of 1215 pigs per treatment. Copper supplementation resulted in an overall improvement of +8.1% for ADG (range-12.0% to +25.2%) and an overall improvement of +5.4% for FC (range-5.2% to +12.6%).

Reports published since 1965 have been conflicting. Berek, Urbanyi and Lakatos (1967), Barber et al. (1968) and Castell and Bowland (1968a) have confirmed the general results outlined in the preceding sections. However, Gipp, Pond and Smith (1967), Bekaert, Eeckhout and Buysse (1967) and Livingstone and Livingston (1968) reported no

response to copper supplementation of the diet at a level of 250 ppm.

3. Effects of dietary copper supplementation on carcass quality

Braude (1965) observed that the majority of reports concerned with copper supplementation of swine diets have indicated no adverse effects on carcass quality, however, a few reports have indicated otherwise. One of the adverse effects on carcass quality attributable to dietary copper supplementation was the occurrence of soft carcass fat. Since this is the subject of the present thesis, the occurrence of soft fat has been discussed separately in a subsequent section of this review.

The addition of copper to swine diets has been reported to adversely affect carcass measurements and grade. Barber et al. (1957) reported that supplementation of diets for fattening pigs with copper at a level of 250 ppm caused a non-significant reduction in the number of carcasses graded triple A in comparison with carcasses of pigs fed the control diet. This was later confirmed. Increased backfat thickness in one experiment (Barber et al. 1961a) and decreased carcass length in another (Barber, Braude and Mitchell, 1960a) were responsible for the loss in market grade.

The observed effects of dietary copper supplementation on dressing percentage, backfat thickness and carcass length have been extremely variable. Allen et al. (1958, 1961) and Barber et al. (1961b) reported a trend towards an increased dressing percentage in pigs fed copper-supplemented diets. Barber et al. (1960a) noted an increase in dressing percentage in one experiment but failed to confirm it subsequently. Similarly, Castell and Bowland (1968a) reported an

increase in dressing percentage in one experiment but in other experiments a decrease in dressing percentage was observed. Wallace et al. (1966b) reported that copper supplementation of the diet did not affect dressing percentage.

Allen et al. (1961) and Barber et al. (1961a) reported that copper supplementation of the diet may increase carcass fat as measured by backfat thickness; but other workers, Barber et al. (1960a, 1961b), Wallace et al. (1966b), Bekaert et al. (1967) and Castell and Bowland (1968a), failed to confirm this observation.

Barber et al. (1960a), Barber et al. (1961a) and Bekaert et al. (1967), reported that copper supplementation of the diet tended to decrease carcass length. Barber et al.(1961b) and Wallace et al. (1966) reported that dietary copper supplementation did not significantly affect carcass length.

Scott, Lewis and Noland (1966) reported that copper supplementation of the diet had a darkening effect on the pork from pigs receiving the supplemented diets.

4. Factors affecting the response to dietary copper supplementation

(a) Identification of copper as the agent responsible for the noted responses

The most common form in which copper is added to swine diets is as copper sulfate ($CuSO_4 \cdot 5H_2O$). Hawbaker et al. (1959) designed an experiment to determine if the copper (Cu^{++}) or the sulfate ($SO_4^{=}$), radical was responsible for the reported improvement in ADG and FC. Three diets, basal, basal plus Na_2SO_4 or basal plus $CuCl_2$ were fed. The pigs fed the diet supplemented with Na_2SO_4 grew at a slower rate

and had poorer FC than those fed the control diet while those fed the diet supplemented with $CuCl_2$ grew faster and had a better FC than the control animals. They concluded that the Cu^{++} radical was the factor responsible for improved performance.

Lucas et al. (1961) supplemented diets with $CuSO_4 \cdot 5H_2O$ in both a commercial and a highly purified form. Similar effects on ADG and FC were observed regardless of purity of the copper sulfate employed. It was concluded that the effect was due to copper per se and not to impurities in commercial grades of $CuSO_4 \cdot 5H_2O$.

(b) Form of copper employed

Several forms of copper, copper sulfate ($CuSO_4 \cdot 5H_2O$), copper chloride ($CuCl_2$), copper sulphide (CuS), copper oxide (CuO), copper carbonate ($CuCO_3$), and copper methionine have been used as supplements in practical swine diets. Data presented by Hawbaker et al. (1959) indicated that $CuCl_2$ added to the ration results in equally as good a response in terms of ADG and FC as $CuSO_4 \cdot 5H_2O$. Barber et al. (1960b) and Barber et al. (1961a) supplemented diets fed to market pigs with $CuSO_4 \cdot 5H_2O$ to supply 250 ppm Cu or CuS to supply 62.5 or 250 ppm Cu. A significant response to copper was observed when pigs were fed the diet containing Cu as $CuSO_4 \cdot 5H_2O$, however, Cu in the form of CuS at either level, had no significant effect on performance. The greater effectiveness of $CuSO_4 \cdot 5H_2O$ as a growth promotant was attributed to its relatively greater solubility in the gut, CuS being highly insoluble (Barber et al. 1960b). Allen et al. (1961) reported that supplementary Cu at a level of 62.5, 125 or 250 ppm as either $CuSO_4 \cdot 5H_2O$ or $CuCO_3$ resulted in improved growth rates in their

experiments. The 250 ppm level of dietary copper gave the best growth rate. Bunch et al. (1964, 1965) reported that copper in the form of $CuSO_4 \cdot 5H_2O$, $CuCO_3$ or Cu-methionine when added to the diet resulted in significantly faster and more efficient gains than were obtained from diets without supplemental copper. $CuSO_4 \cdot 5H_2O$ and Cu-methionine promoted a better FC than did $CuCO_3$. Bunch et al. (1960, 1961, 1963) reported that copper in the form of CuO fed at a level to provide 250 ppm supplemental copper was equally as effective as $CuSO_4 \cdot 5H_2O$ as a growth promotant in swine diets. However, Wallace et al. (1962) reported that growing-finishing pigs did not respond to copper at a level of 250 ppm either as $CuSO_4 \cdot 5H_2O$ or CuO. Copper oxide depressed gains in this case.

It can be concluded that copper in several forms, excluding CuS can be effective in promoting ADG and for improving FC. There is, however, a reason for the common usage of $CuSO_4 \cdot 5H_2O$. Braude (1965) states that: "We recommend that copper sulphate be used in preference because this compound has a bitter taste, and pigs will refuse to consume rations containing copper in this form considerably in excess of the recommended level of 250 ppm of Cu."

(c) Level of copper

A large number of experiments have been carried out to determine the optimum level of dietary copper supplementation required to maximize ADG and improve FC. Levels of supplemental copper used in such experiments have ranged from 0 to 1250 ppm (0 to 0.5% $CuSO_4 \cdot 5H_2O$ in the diet). The majority of such reports, Barber et al. (1957); Hawbaker et al. (1959, 1961); Bunch et al. (1960, 1961, 1964, 1965);

Lucas et al. (1961); Fagan et al. (1961); Allen et al. (1961) and Wallace et al. (1962), have supported the contention that the optimum level of dietary copper supplementation is 250 ppm (0.1% $CuSO_4 \cdot 5H_2O$ in the diet). When Wallace, Hauser and Combs (1966a) offered pigs simultaneous access to meal mixtures containing 0, 125, 250, 500 and 1000 ppm of supplemental copper; the pigs ate more of the meal mixture with no supplemental copper and ate progressively less of the various mixtures as copper level increased, an observation which contradicts that made by Mitchell (1953).

(d) Copper toxicosis

When dietary copper levels were in excess of 250 ppm, decreased performance was observed (Barber et al. 1956, 1957; Lucas and Calder, 1957a; Wallace et al. 1960; Bunch et al., 1965) and copper toxicosis sometimes occurred (Wallace et al. 1960). It has also been reported occasionally that copper toxicosis may occur even when a level of 250 ppm of copper was added to the diet (Wallace et al. 1960; Bass et al. 1956). Other reports of copper toxicosis have usually involved a limited number of animals or extremely high levels of dietary copper supplementation (Gordon and Luke, 1957; Allcroft, Burns and Lewis, 1961; Buntain, 1961; Allen and Harding, 1962; Hemingway, 1962). However, the results of these latter studies have served to describe the symptoms of copper toxicosis.

There are indications that the response to copper and the occurrence of copper toxicosis can be affected by the type and level of protein, and the presence and levels of certain other trace minerals in the diet (Wallace et al. 1960; Suttle and Mills, 1966a,

1966b; Hanrahan and O'Grady, 1968; O'Donovan, Spillane and O'Grady, 1966).

(e) Composition of the diet

1. Type and level of protein

The early studies on copper supplementation of the diet were carried out in Great Britain where fishmeal (FM) is a common protein supplement in practical swine diets. Studies in the United States where soybean meal (SM) is the protein supplement generally used did not demonstrate the same response. This indicated that the type of protein supplement, animal versus vegetable, used in the diet might affect the response to dietary copper supplementation. In addition, studies on the occurrence of copper toxicosis (section A-4-d) indicate that level of protein might also exert an effect on the response to added dietary copper.

Wallace et al. (1960) supplemented corn-soybean meal diets with copper at a level of 100, 150, 200 or 250 ppm. The level of 250 ppm proved toxic in a few cases and consistent growth responses and improved FC were not observed at any level of supplemental copper. Allen et al. (1961) reported that when copper at a level of 250 ppm was added to diets containing either FM or dried skim milk as protein sources, response in terms of ADG, FC and feed intake, was greater in diets supplemented with dried skim milk than in diets supplemented with FM. Barber, Braude and Mitchell (1962) reported that the response to a copper supplement was greater in diets containing FM than in diets containing SM: ADG +14.5% versus +5.1% and FC +5.2% versus +2.1%, respectively. However, Lucas, Livingstone and Boyne (1962) found

that there was no evidence of a different effect of copper supplementation of barley-fishmeal diets as compared to corn-soybean meal diets provided the level of protein in the diet was adequate. Combs et al. (1966) reported that response to copper supplementation of the diet in terms of ADG was similar regardless of protein source in the diet; SM or casein. Similarly, O'Donovan, Spillane and O'Grady (1966) reported no significant differences with respect to ADG and FC when either FM, SM or dried skim milk were the protein sources in diets supplemented with copper. On the other hand, however, Castell and Bowland (1968a) reported that the beneficial effects of copper were more evident in pigs fed FM than in pigs fed SM.

Level of protein may also affect the response to dietary copper supplementation but results are conflicting. Bunch et al. (1961) reported that the response to copper was similar in corn-soybean meal-fishmeal diets containing either 16 or 22% protein. Level of protein (14 versus 22%) as either SM or casein did not affect the response to copper supplementation of the diet (Combs et al. 1966). King (1964) reported that in barley-fishmeal diets with a low (14.5%) protein level, copper supplementation resulted in improved ADG and FC as compared with the basal diet. This response was not observed with the high (18.1%) protein diet. There were no significant differences in ADG between the high and low protein diets supplemented with copper. Beames and Lloyd (1964) reported that copper supplementation of swine diets containing a low level of SM (6%) resulted in improved ADG while supplementation of diets containing a high level of SM (23%) did not affect body weight gain and tended to reduce feed consumption. Castell and Bowland (1968a) reported that the beneficial effects of

copper supplementation were more evident when diets of relatively high protein content were fed. Hanrahan and O'Grady (1968) reported that the performance of pigs fed a low protein diet supplemented with copper was significantly reduced while that of pigs fed a high protein, copper-supplemented diet was only slightly reduced.

Level of protein may influence body stores of copper. Wallace et al. (1960) reported that as dietary protein level increased from 15% to 25% the toxic effect of 750 ppm dietary copper became less as measured by ADG, FC and blood hemoglobin level. McCall and Davis (1961), using the rat and Combs et al. (1966), using the pig, have reported that as protein level in the diet increases storage of copper in the liver decreases. This may influence the response of pigs to dietary copper supplementation.

2. Mineral balance

There is increasing evidence which indicates that the response to copper supplementation and the occurrence of copper toxicosis may be affected by the levels of other minerals, especially zinc, present in the diet. Some workers, Wallace et al. (1960) and McCall and Davis (1961) have reported no apparent relationship between copper and zinc when diets supplemented with either copper, zinc or both these elements were fed. Other workers, notably Hanrahan and O'Grady (1968), have reported that there was no reduction in performance on low protein diets and a greater improvement in performance on high protein diets when zinc as well as copper supplements were added. Concomitant zinc supplementation eliminated the toxicosis associated with copper supplementation at either protein level and reduced liver copper accumulation.

Suttle and Mills (1966a,b) also reported that the addition of zinc to a diet of high copper content decreased the incidence of copper toxicosis.

3. Mode of action of copper

(a) Similarity of response to copper to that obtained with antibiotics

Barber, Braude and Mitchell (1955b) noted that the effects of dietary copper supplementation appeared to be similar to those of antibiotic supplements. Diets were supplemented with a mineral mix (containing copper), Aurofac, Aurofac plus the mineral mix or $CuSO_4 \cdot 5H_2O$. All treatments were equally effective in promoting a significantly improved rate of gain as compared with the control diet. Similarly, Barber et al. (1957) reported that supplementation of diets with either $CuSO_4 \cdot 5H_2O$ (0.1%), terramycin (10 g/ton) or aureomycin (20 g/ton) resulted in an increased rate of gain and an improved FC as compared to the animals fed the control diet. Hawbaker et al. (1959) and Bunch et al. (1961) have reported similar results. It was not always clear whether the improved rate of gain was associated with increased feed consumption or with more efficient feed utilization.

Other workers, Lucas and Calder (1957b), Hawbaker et al. (1959) and Barber et al. (1960a) have reported that the effect of copper and antibiotics may be additive. In some experiments, when diets were supplemented with copper and an antibiotic greater responses were obtained than with diets supplemented with either alone. Bellis (1961) and Braude et al. (1962) were unable to confirm this observation while Hawbaker et al. (1961) reported that the combination of $CuSO_4 \cdot 5H_2O$ and

several antifungal agents gave no greater response than $CuSO_4 \cdot 5H_2O$ alone.

(b) Effect of copper on the intestinal flora of the pig

It was thought that copper might exert its growth promoting effect by influencing the kinds and numbers of organisms present in the intestinal tract (Hawbaker et al. 1959). Fuller et al. (1960) reported that copper resulted in a reduction in the numbers of streptococci with no change in the numbers of lactobacilli or E.coli present in the feces of pigs fed diets supplemented with copper sulfate. Bunch et al (1961) and Hawbaker et al. (1961) reported that supplementation of swine diets with $CuSO_4 \cdot 5H_2O$ resulted in lowered fecal counts of lactobacilli, total aerobes and total anaerobes, with increases in numbers of molds, yeasts and E.coli. Smith and Jones (1963) studied the flora in various sections of the gastrointestinal tract (GIT) of pigs fed a diet supplemented with $CuSO_4 \cdot 5H_2O$ and compared it with the flora present in the GIT of pigs fed a basal diet. There were no changes in the kinds and numbers of bacteria present in the various sections of the GIT which could be attributed to copper supplementation of the diet. They concluded that their results did not support the view that the action of copper is similar to that of antibiotics. The experiment of Smith and Jones (1963) was performed on 75 kg pigs which presumably would have an established intestinal flora. The experiments of Bunch et al. (1961) and Hawbaker et al. (1961) employed relatively young pigs which may have had a different intestinal flora.

4. Accumulation of copper in porcine tissues

Tissue copper concentrations are highest in the liver, heart,

kidneys, hair and brain, lower in the spleen and bones and lowest in the endocrine glands; however, only the liver, kidney, spleen and lungs are susceptible to changes in copper concentration resulting from changes in dietary copper intake (Underwood, 1962).

Barber et al. (1957, 1961a) studied the distribution of copper in a wide variety of tissues from pigs fed either basal diets or the basal diet supplemented with $CuSO_4 \cdot 5H_2O$. Copper supplementation of the diet resulted in accumulation of copper mainly in the liver and kidneys with no significant change in copper levels in the heart, spleen or backfat. In one experiment, (Barber et al. 1957) supplemental copper resulted in an increase of copper concentration in the backfat from 0.66±.27 mg Cu/kg wet tissue in the basal pigs to 1.30 ± .27 mg Cu/kg wet tissue in the copper supplemented pigs; however, this increase was not noted in a further experiment (Barber et al. 1961a). Bowland et al. (1961) studied the distribution of orally administered Cu^{64} and found that 24 hours after administration the accumulation of Cu^{64} was highest in the liver and kidneys and relatively low in all other tissues studied including backfat. Similar results have been reported by others for example, Dammers and Van der Grift (1963), Castell and Bowland (1968b).

B. FATTY ACID COMPOSITION OF PORCINE DEPOT FAT

1. Constituent fatty acids

Hilditch and Williams (1964) state that "with pigs raised on diets relatively low in fat the major component depot fatty acids are palmitic acid (16:0)[1] stearic acid (18:0) and unsaturated 18 carbon

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[1](Number of carbon atoms: number of double bonds in molecule).

acids in which oleic acid (18:1) predominates". The other 18 carbon unsaturated acids are linoleic acid (18:2) and, linolenic acid (18:3). Together these major fatty acids account for greater than 90% of the fatty acid composition of porcine depot fat. This has been amply confirmed by others (deMan and Bowland, 1963; Hubbard and Pocklington, 1968).

In addition to the major fatty acids, Hilditch and Williams (1964) list several fatty acids as minor components of porcine depot fat: myristic acid (14:0), tetradecenoic acid (14:1), palmitoleic acid (16:1), 20 carbon unsaturated acids as well as trace of capric acid (12:0). Stinson (1966) has suggested that arachidic acid (20:0) might also be a minor component of porcine depot fat.

Specific values for the fatty acid composition of porcine depot fats cannot be given as this composition can be altered by a variety of factors to be discussed in the following sections. However, Leat et al. (1964) have described the fatty acid composition of several depot fats from pigs fed a semi-purified fat-free diet. Such data represent the fat composition resulting when the body fat is derived entirely from endogenous synthesis by the animal.

2. Effect of area sampled

The fatty acid composition obtained on analysis of the depot fat of pigs can be markedly affected by the area from which the fat sample is taken. Hilditch and Williams (1964), Sink et al. (1964) and Stinson, deMan, and Bowland (1967) have all reported on the effect of sampling site on the fatty acid composition of porcine depot fat. There is general agreement among these authors that the outer backfat

layer contains a lower total percentage of the saturated fatty acids (14:0, 16:0, 18:0) and a higher total percentage of the unsaturated fatty acids (16:1 and 18:1). The leaf or perinephric fat contains a higher percentage of saturated acids than the backfat concomitant with a decrease in the percentage of unsaturated acids. However, 18:2 maintains a fairly constant proportion regardless of sampling site (Hilditch and Williams, 1964). The fatty acid composition at a site may be related to the body temperature at that site; the outer fat layers having a lower temperature also contain greater porportions of unsaturated fatty acids which have a lower melting point. As one proceeds inwards the body temperature increases and the proportion of saturated fatty acids having a higher melting point also increases. This relationship is discussed by Hilditch and Williams (1964). More recently MacGrath et al. (1968) using iodine value as a measure of the degree of unsaturation in a fat sample showed that as body temperature increased the iodine value of the fat decreased.

Data presented by Sink et al. (1964) and Stinson et al. (1967) indicates that the differences in fat composition between major fat depots are greater than differences in fat composition between anatomical areas. For example, one would find a greater difference in composition between samples taken from the outer and inner backfat layers at the shoulder than would exist within samples of either outer or inner backfat taken at the shoulder, loin or rump.

3. Effects of diet

It is not the purpose of this literature review to review the details of the digestion, absorption and deposition of dietary fat,

however, in order to orient the reader, a few comments on this subject seem appropriate. The following discussion is based on reviews by Senior (1964) and Shapiro (1967).

Dietary fat is hydrolyzed, by the action of lipase secreted into the GIT, with the aid of the bile. Subsequently micelles composed largely of monoglycerides, free fatty acids and bile salts are presented to the surface of the intestinal mucosa. The monoglycerides and free fatty acids pass into the cells of the intestinal mucosa where a division of the fatty acids occurs. Fatty acids consisting of 12 or less carbon atoms pass largely into the portal circulation and are thus carried to the liver. Other fatty acids containing 14 or more carbon atoms are resynthesized into triglycerides either by recombination with absorbed monoglycerides or with glycerol derived from glucose. These reconstituted triglycerides are combined with lipoprotein to form chylomicrons. The chylomicrons pass into the lymph ducts and are transported via the lymph ducts to the bloodstream. Once in the bloodstream they are rapidly removed by adipose tissue, heart, muscle and liver. On reaching a tissue and being taken up by this tissue the triglycerides are purported to be hydrolyzed. Component fatty acids are used for the synthesis of new triglycerides or are oxidized to form CO_2 and H_2O. From the foregoing discussion one can see that since certain fatty acids can pass, by the described processes, from the diet to the depot fat virtually unchanged, the composition of the depot fat can be influenced by the composition of the dietary fat. The degree of influence would of course depend on the level of fat in the diet as pointed out by Hilditch and Williams (1964) or on the absolute amount of lipid consumed during the experimental period

(Dahl and Persson, 1965).

Leat et al. (1964) compared the fatty acid composition of depot fat from pigs fed a diet containing no added fat, 10% maize oil or 10% tallow. In the pigs fed no fat the major component acids in the depot fat were 18:1 (55%), 16:0 (24%) and 18:0 (13%) while the concentration of 18:2 was less than 1%. When maize oil was added to the diet the 18:2 concentration of the depot fat rose to 25-30% at the expense of 18:1, 16:0 and 18:0. The addition of tallow to the diet had little effect on the composition of the depot fat. Similar results were obtained when corn oil or tallow were added to a diet containing no added fat by MacGrath et al. (1968).

Feeding diets supplemented with 10% safflower oil to pigs resulted in decreased amounts of 16:0 and 18:1 in the depot fat compared with the depot fat of pigs fed the same diet with no added fat. Changing the pigs from a safflower oil supplemented diet to one supplemented with 10% tallow reversed this trend and resulted in decreased 18:2 concentration and increased concentrations of 16:0 and 18:1 (Koch et al. 1966, 1968b). The feeding of soybean oil, Brooks (1967) at a level 10% to market pigs almost tripled the 18:2 content of the depot fat in comparison with control animals fed a basal diet with no added fat, 31.7% versus 12.1%, respectively. The increase in 18:2 was at the expense of 16:0, 18:0 and 18:1.

The feeding of fats containing high proportions of unsaturated fatty acids such as corn oil, soybean oil or safflower oil exerts a marked effect on the fatty acid composition of porcine depot fats. Transferring pigs so fed to a diet containing a fat which contains a high proportion of saturated fatty acids such as tallow will reverse

the trend tending to make the depot fat more saturated.

4. Effect of feeding system: ad libitum versus restricted

Iodine number, refractive index, and melting point can all be used to give an indication of the degree of unsaturation (or softness) of a fat. Iodine number and refractive index increase while melting point decreases as the proportion of unsaturated fatty acids increases (Kirschenbauer, 1960).

Ellis and Zeller (1931a) reported that as the level of feeding of swine decreased from full-feeding to 50% of full-feeding both the refractive index and the iodine number of the depot fat increased. The increase in refractive index of the depot fat with decreases in level of feeding was confirmed by Shorrock (1940). Babatunde et al. (1967) pair-fed pigs from 45 kg to slaughter at 79, 90 or 102 kg either ad libitum or 1.82 kg feed per day. Restricting feed intake to 1.82 kg per day significantly increased the percentages of the unsaturated fatty acids and decreased the percentages of the saturated fatty acids as compared to the percentages present in the carcasses of pigs fed ad libitum. Similarly Greer et al. (1965) reported that as the level of feed intake allowed pigs was decreased from full hand-fed to 70% of full hand-fed the percentage of total unsaturated fatty acids in the outer layer of backfat increased with a concomitant decrease in the percentage of total saturated fatty acids.

Friend and Cunningham (1967) pair-fed pigs so that one animal in each pair was allowed to eat to appetite for 30 min each day while its littermate was given the same amount of feed over five feedings daily. Pigs fed five times daily produced a fat which had significantly

less 18:1 than did the fat of pigs fed the same amount of diet once-daily.

Several groups have reported that the energy level of the diet will exert an effect on the fatty acid composition of porcine depot fat. Clausen and Ludvigsen (1960) concluded, after surveying the literature, that when the daily energy intake of the pig was restricted by adding a "filler" to the diet a softer backfat resulted. Lucas, McDonald and Calder (1960) compared the iodine value of the depot fat of pigs fed at a very high (VH) plane of nutrition to 45.5 kg liveweight, at which time a number of the pigs were switched to a very low (VL) plane of nutrition. Pigs fed at the VH-VH plane produced a backfat which had a lower iodine value (62.5 for the inner backfat and 66.5 for the outer backfat) than did pigs fed at the VHVL plane of nutrition (67.3 for the inner backfat and 71.7 for the outer backfat). Koch, Parr and Merkel (1968a) found in one experiment that dietary energy levels below 80% of full-feed significantly decreased the 18:0 content and increased the 18:1 content of porcine backfat. However, in a second experiment the fatty acid composition of the backfat from pigs fed 80% of full-feed was not significantly different from that of full-fed pigs.

From the data presented, it would appear that level of feeding and energy content of the diet can exert a significant effect on the fatty acid composition of porcine depot fat.

5. Effect of sex

Friend and Cunningham (1967), reported that the backfat of gilts contained lower proportions of saturated and higher proportions of unsaturated fatty acids than did that of barrows. This was in contradiction to the report of Babatunde et al. (1967) who found no

significant differences in the fatty acid composition of backfat samples from barrows and gilts. Koch et al. (1968a) noted that the backfat of spayed gilts contained significantly less 18:2 than that from boars or barrows while the backfat of boars had significantly greater amounts of this acid than that of either barrows or gilts. In one experiment the backfat from barrows contained significantly greater amounts of 18:0 than that of gilts.

Allen, Bray and Cassens (1967) determined the fatty acid composition of lipid obtained from the longissimus dorsi muscle of the pig. This study involved 30 pigs slaughtered at two liveweights (49.6 and 94.1 kg). It was reported that the effects of sex on fatty acid composition were restricted to the triglyceride fraction of the muscle lipids. The triglyceride fraction of the muscle lipid from heavy barrows and gilts contained a greater proportion of 18:0 and a lower proportion of 18:1 than did the triglyceride fraction of the muscle lipid from heavy boars.

6. Effect of liveweight

Elson et al. (1963), Allen and Bray (1964), Sink et al. (1964), Allen et al. (1967) have all reported that as liveweight increases the intramuscular and depot fat of pigs becomes more saturated, ie., the proportion of saturated fatty acids increased while the proportion of unsaturated fatty acids decreased with increasing liveweight.

C. ENDOGENOUS SYNTHESIS OF FATTY ACIDS

When diets consisting of normal feedstuffs, and, therefore,

relatively low in fat are fed, it is unlikely that the fatty acid composition of the diet will appreciably affect the fatty acid composition of the animals depot fat. It must be assumed, under such conditions, that the fatty acid composition of the depot fat is a reflection of the animals endogenous capacity to synthesize fatty acids. Therefore, it seems appropriate to briefly outline the present knowledge concerning the pathways of de novo fatty acid synthesis in animals. This discussion is based on recent reviews by Wakil (1964), Elovson (1966), Olson (1966), Shapiro (1967), Lynen et al. (1967) and Masoro (1968).

1. De novo synthesis of fatty acids

The enzymatic machinery for fatty acid synthesis is located in the cytoplasm of cells at a site other than the mitochondria or microsomes. The primary product synthesized in vitro is 16:0 although small amounts of 14:0 and 18:0 may also be formed. A second site of fatty acid synthesis is located within the mitochondria and produces mainly 18:0. However, there is some doubt as to whether the mitochondrial system does in fact accomplish de novo synthesis or merely elongates the 16:0 produced by the cytoplasmic system.

De novo synthesis in the cytoplasm is the result of combining acetyl-CoA and malonyl-CoA according to the following equation:

$$1\ \text{Acetyl-CoA} + 7\ \text{Malonyl-CoA} + 14\ \text{NADPH} + 14\ H^{+} \xrightarrow[\text{synthetase}]{\text{fatty acid}}$$

$$16{:}0 + 14\ \text{NADP} + 7\ CO_2 + 8\ \text{HSCoA} + 6\ H_2O$$

The fatty acid synthetase which accomplishes the condensation is a multienzyme complex to which the intermediates are bound by a thioester linkage to acyl-carrier protein throughout the reaction. The product of the reaction in animals undergoes deacylation to be released from the enzyme complex as the free acid. The product of this synthesis 16:0, is as pointed out earlier, a major component of the depot fat of the pig.

2. Elongation of fatty acids

The product of fatty acid synthesis, 16:0, can be elongated to form higher fatty acids by one or other of two fundamentally different systems. Both systems, however, require activation of the fatty acid to its fatty acyl-CoA form.

The first system is associated with the microsomal fraction of cells and elongates fatty acids according to the following equation:

$$\text{Palmityl-CoA} + \text{Malonyl-CoA} + 2\ \text{NADPH} + 2\ H^{+} \longrightarrow \text{Stearyl-CoA} + 2\ \text{NADP} + CO_2$$

This system can elongate both saturated and unsaturated fatty acids and thereby produce a wide variety of fatty acids. The highest saturated fatty acid produced by this system in animal tissues is lignoceric acid (24:0).

The second system for the elongation of fatty acids is located within the mitochondria of cells and accomplishes elongation according to the following equation

$$\text{Palmityl-CoA} + \text{Acetyl-CoA} + 2\ \text{NADPH} + 2\ H^{+} \longrightarrow \text{Stearyl-CoA} + 2\ \text{NADP}$$

Little is known regarding the relative importance of these two systems for the elongation of fatty acids or the enzymology of the systems. However, 18:0 the second most abundant saturated fatty acid found in animal fats is produced in large measure by the elongation of 16:0.

3. Desaturation of fatty acids

The major site of fatty acid desaturation is associated with the microsomal fraction of cells. The system requires that the substrate for desaturation be in the form of its acyl-CoA ester, however, the activation of a fatty acid to its acyl-CoA form is accomplished in the cytoplasm. This system cannot introduce a double bond between C-9 and the methyl end of a fatty acid molecule, hence any fatty acids found in the animal body with a double bond between C-9 and the methyl end are considered essential. For example, linoleic acid (18:2, octadeca-9, 12-dienoic acid), linolenic acid (18:3 octadeca-9, 12, 15-trienoic acid) and arachidonic (20:4, eicosa-5, 8, 11, 14-tetraenoic acid). However, 20:4 can be synthesized in the animal body by elongation and desaturation of 18:2.

The endogenous synthesis of 16:1 and 18:1, the major unsaturated fatty acids found in animal lipids proceeds according to the following equations:

$$\text{Palmityl-CoA} \xrightarrow{NADPH_2 + O_2} \text{Palmitoleyl-CoA (16:1)}$$

$$\text{Stearyl-CoA} \xrightarrow{NADPH_2 + O_2} \text{Oleyl-CoA (18:1)}$$

Little is known concerning the enzymology of this system, however, the

animal can, by combining this system with the system for the elongation of fatty acids, produce a wide variety of unsaturated fatty acids of varying chain lengths. Figure 1, taken from Wakil (1964), summarizes the metabolic capabilities of the animal with respect to fatty acid synthesis.

4. Site of fatty acid synthesis

Fatty acid synthesis occurs to a greater or lesser extent in most animal tissues; however, little is known concerning the major site of de novo fatty acid synthesis. Some workers feel that the adipose tissue is the most active site while others feel that the liver or gastrointestinal tract are major sites. It appears, however, that the major site of fatty acid desaturation and elongation is located in the liver (Masoro, 1968).

D. EFFECTS OF DIETARY COPPER SUPPLEMENTATION ON PORCINE DEPOT FAT

Reports have appeared in the literature indicating that dietary copper supplementation may influence porcine fat composition. Taylor and Thomke (1964) and Thomke and Taylor (1964) reported that the supplementation of diets fed to market pigs with 250 ppm Cu resulted in a small but significant increase in the iodine number (Hanus) of the backfat. Bekaert et al. (1967) reported a similar trend. There was a significant decrease in the percentage of stearic acid and a significant increase in the percentage of oleic acid present in the backfat of pigs fed the copper supplemented diets as compared to the control animals. Bowland and Castell (1965) reported a greater incidence of soft fat in pigs fed copper supplemented diets. More recently, Moore et al. (1968) have

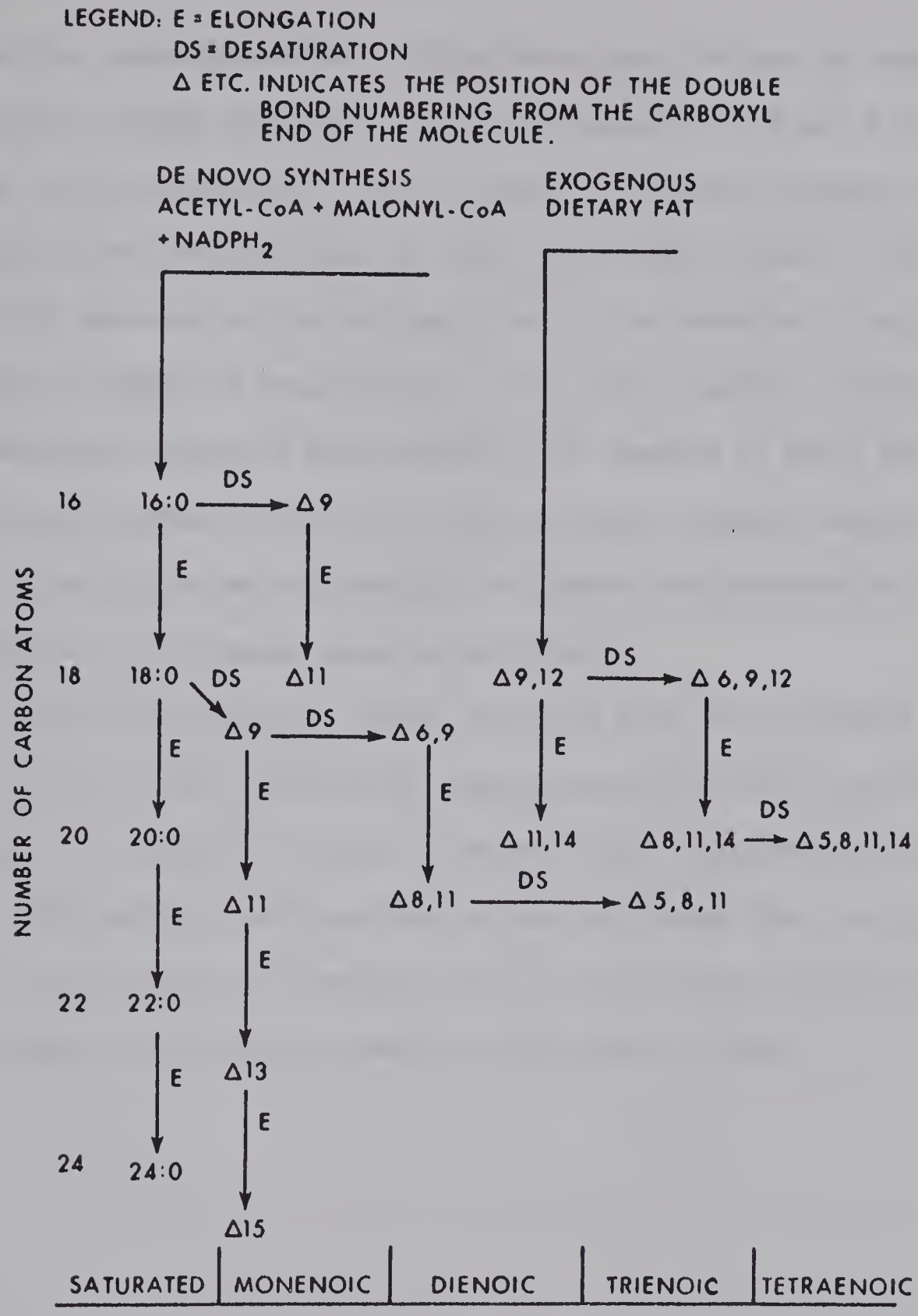

Figure 1. A summary of the metabolic pathways involved in fatty acid synthesis in animals.

reported that supplementation of pigs diets with 250 ppm of copper resulted in a slight increase in the percentage of 18:1 and a slight decrease in the percentage of 18:0 present in the whole backfat with no change in the concentration of other constituent acids. There was also a 10°C decrease in the melting point of the backfat of pigs fed supplemental copper as compared with the control animals. Further experimentation indicated that although the changes in fatty acid composition occurred in both the inner and outer backfat layers, the change in melting point attributable to copper supplementation of the diet occurred in the inner backfat layer only.

Castell and Bowland (1968a) reported that the incidence of soft carcass fat was lower when barley-soybean meal diets were supplemented with copper as compared to barley-fishmeal diets supplemented with copper. Furthermore, soft carcass fat was not noted when the pigs were fed to a scale based on liveweight but its occurrence was quite marked amongst pigs fed ad libitum (Castell and Bowland, 1968a).

GENERAL EXPERIMENTAL

A. MANAGEMENT OF EXPERIMENTAL ANIMALS

All pigs used in this work were farrowed at The University of Alberta Livestock Farm and were of Hampshire × Yorkshire breeding. The animals were weaned at either three (Experiment 1) or five (Experiment 2) weeks of age, and placed directly on the experimental diets.

The following routine management practice for all pigs in The University of Alberta swine herd applied in the present case. All pigs were identified by ear notch at birth and "black" teeth were removed. A 2 ml injection of Pigdex-100[1] (an iron dextran compound containing 100 mg Fe/ml) was given to each animal at approximately 4 days of age to prevent anemia. Male pigs were castrated at 10 days of age. All pigs were treated with lindane[2] at 5 to 6 weeks of age to prevent mange. A second lindane treatment was given 10 days later. Pigs were vaccinated at approximately 8 weeks of age with 5 ml of erysipelas bacterin[3] to prevent erysipelas. Between 8 and 12 weeks of age the pigs were fed a diet containing Dowzene DHC[4] to eliminate ascarids.

The pigs were confined in pens which allowed individual feeding. Water was available free choice in automatic waterers. Diets were mixed at The University of Alberta Livestock Farm.

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[1]Cyanamid of Canada Ltd., Agricultural Products, Montreal, P.Q.

[2]Gamma-benzene hexachloride.

[3]Agricultural Division, Chas. Pfizer and Co., Inc., New York, N.Y.

[4]Dow Chemical of Canada Ltd., Toronto, Ontario.

B. COLLECTION AND HANDLING OF SAMPLES

1. Feed samples

Samples of feed for determination of dry matter, gross energy, crude protein and crude fat were obtained at the time of mixing with the aid of a seed sampler[1]. One sample was taken from each bag in the mix. The samples were pooled prior to sampling for analysis.

2. Fecal samples

Fecal samples were obtained three times a day; 8:00 a.m., 11:30 a.m., and 5:00 p.m. for two consecutive days. These were pooled, placed in plastic bags and stored at -35°C. Prior to analysis, the fecal samples were weighed, dried in a forcedair oven[2] for 48 hr at 60°C, allowed to equilibrate with the air for a further 48 hr before final weighing. Subsequently, the samples were ground in a C and N Laboratory Mill[3], to pass through a 2mm mesh screen and were analyzed for dry matter, nitrogen, energy and chromic oxide as outlined under section D.1.

3. Fat samples

In Experiment 1, fat samples were obtained at slaughter. A strip of backfat, approximately 2.5 cm wide, was taken from the right half of the carcass, adjacent to the midline, from shoulder to rump.

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[1]Seedboro Equipment Co., Chicago, Illinois, U.S.A.
[2]Style v31, Despatch Oven Co., Minneapolis, Minnesota, U.S.A.
[3]Size 8, Christy and Norris Ltd., Chelmsford, England.

A pooled random sample of the perinephric fat from both the right and the left sides of the carcass was also obtained. These were stored in glass bottles under N_2 at -20°C.

During the preliminary stages of Experiment 2, an attempt was made to use the biopsy needle described by Diengott and Kerpel (1967). The use of this needle, either directly or by first making a small incision through the skin, caused excessive bleeding resulting in sample contamination and was apparently very painful to the animal.

During the actual experiment, fat samples were obtained by needle biopsy using a method described by Hirsch et al. (1960). This method was modified slightly in that a 16-gauge needle and a 20 ml syringe rather than a 18-gauge needle and a 50 ml syringe were used. The needle was inserted under the skin adjacent to the midline and just back of the right shoulder. The sample so obtained was transferred from syringe to sample bottle with a 2:1 (v/v) mixture of $CHCl_3$:CH_3OH. When several pigs were to be sampled a separate syringe was used for each pig. Samples were stored in CH_3Cl_3:CH_3OH under N_2 at -35°C. The amount of lipid material obtained for analysis by this method varied with the liveweight of the animal sampled. Sixty-four animals were sampled at average liveweights of 34.4, 45.4, 57.0, 68.0 and 78.9 kg and the average amount of lipid obtained was 33.7, 63.2, 69.7, 74.1 and 75.4 mg, respectively. These amounts were sufficient for analysis by gas-liquid chromatography.

C. LIPID ANALYSIS

1. Depot fat samples

(a) Extraction of lipids

In Experiment 1, lipids were extracted from the fat samples with petroleum ether (bp 39.5 - 49.4°C) for 24 hrs on a Goldfish apparatus[1]. The long extraction time and limited equipment led us to seek a quicker means of extracting lipids.

The method of Folch et al. (1957) was decided upon because a large number of samples could be extracted by this method in a short period of time with the equipment available. Since it was desirable to be able to compare the results from both experiments, and since % composition of the lipid sample was the parameter being compared, it was necessary to examine the % composition of samples of the same material extracted by these two methods. A sample of perinephric fat from a pig was obtained at a local slaughter house. Triplicate extractions were performed by the aforementioned methods. The lipids so obtained were prepared as described in the discussion to follow, chromatogrammed in duplicate and their weight % composition determined. Since there was greater variation in weight % composition of samples extracted by the same method than existed between methods, it was concluded that the results from samples extracted by either method were comparable. The method of Folch et al. (1957) was used to extract samples obtained in Experiment 2. In using this method for backfat samples obtained at slaughter, a 1 g sample was homogenized

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[1]Laboratory Construction Co., Kansas City, Missouri, U.S.A.

with 20 ml of $CHCl_3$:CH_3OH (2:1) in a Virtis "23" homogenizer[1]. The homogenizing vessel was surrounded by ice. When the samples were obtained by needle biopsy the homogenization step was eliminated from the procedure.

(b) Preparation of methyl esters

Methyl esters were prepared from the extracted lipids by transesterification according to the method of Morrison and Smith (1964) for triglycerides. n-Pentane replaced benzene in this method. The lipid extracts were first filtered through anhydrous Na_2SO_4 to remove any water, the solvent removed under N_2 on a flash evaporator[2] and approximately 50 mg of the lipid so obtained transferred under N_2 to a 150 × 20 mm screw cap culture tube[3] for methanolysis.

(c) Gas-liquid chromatography

Each sample of the methyl esters was chromatogrammed in duplicate in Experiment 1 and singly in Experiment 2 using an Aerograph model A90-P3 gas chromatograph[4] equipped with a thermal conductivity detector. Separation of the methyl esters was achieved on a 180 × 0.62 cm aluminum column packed with 20% diethylene glycol succinate on 60-80 mesh firebrick. The following isothermal conditions prevailed: column temperature 210°C, injector and detector temperature 250°C, helium flow rate 100 ml. per min., filament current 200 ma.

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[1]The Virtis Company, Yonkers, N.Y., U.S.A.

[2]Rinco Instruments Co. Inc., Greenville, Illinois, U.S.A.

[3]Cat. No. 9826. Corning Glassworks. Fisher Scientific. Edmonton.

[4]Varian Aerograph. 2700 Mitchell Drive, Walnut Creek, California, 94598, U.S.A.

(d) Identification of acids

Individual fatty acids were identified by comparing their retention times with the retention times of pure fatty acid methyl esters. The following fatty acids were identified 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0[1]. Several other minor components were present, however, these were not identified. The acids 14:0, 16:0, 16:1, 18:0, 18:1 and 18:2 together accounted for more than 95% of the fatty acids in the samples.

(e) Methods of calculation.

1. Calculation of peak areas

Peak areas were obtained by planimetry in the case of the outer and inner backfat samples in Experiment 1. In the case of the perinephric fat in Experiment 1 and all subsequent samples, peak areas were obtained with the aid of an electronic integrator[2] attached to the recorder[3].

2. Calculation of percent composition

Correction factors for detector response were obtained by daily chromatogramming a standard sample of known weight % composition which approximated the composition of porcine depot fat. The percentage composition of the standard sample was determined from the peak areas and correction factors obtained by comparing these figures with the

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[1] 14:0 myristic acid; 16:0 palmitic acid; 16:1 palmitoleic acid; 18:0 stearic acid; 18:1 oleic acid; 18:2 linoleic acid; 18:3 linolenic acid; 20:0 arachidic acid.

[2] Integraph Integrator Model 49. Photovolt Corporation. 1115 Broadway, New York, N.Y., 10010, U.S.A.

[3] Microcord Model 44. Photovolt Corporation. 1115 Broadway, New York, N.Y., 10010, U.S.A.

known weight % composition of the sample. All correction factors derived for a given acid on a given column were averaged and applied to chromatograms run on that column to arrive at corrected peak areas. The corrected areas were totalled and the individual areas expressed as a percentage of the total to arrive at weight % composition.

(f) Determination of melting point

The melting point of all samples in Experiment 1 was determined. The samples had been extracted as previously described (section C1. (a)) and the melting points were determined by the Wiley method (AOAC, 1965). Only single determinations were carried out on each sample.

D. OTHER CHEMICAL ANALYSIS

1. Nitrogen

The nitrogen content of feed and feces was determined by the Kjeldahl method of analysis (AOAC, 1960). A commercial Kel-Pak[1] was used to provide the required amount of catalyst for the acid digestion. The ammonia ultimately produced was retained in a 4% boric acid solution. Protein level was calculated by multiplying nitrogen x 6.25.

2. Gross energy

Gross energy of the feed was determined using a Parr Oxygen Bomb Calorimeter[2] equipped with a Brown Electronik recorder[3].

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[1]Matheson Scientific, East Rutherford, New Jersey. Supplies Hg catalyst, K_2SO_4 and $CuSO_4$.

[2]Parr Instrument Company. Moline, Illinois, U.S.A.

[3]Minneapolis - Honeywell Regulator Company. Philadelphia, Pennsylvania, U.S.A.

3. Dry matter

Dry matter determinations on feed and fecal samples were performed by drying a sample of known weight in a forced air oven[1] for 24 hr at 110°C. The samples were then cooled in a dessicator until constant weight was obtained.

4. Chromic oxide

The chromic oxide content of feed and fecal samples was determined using the method of Hill and Anderson (1958). Twenty ml of concentrated H_2SO_4 was added to the flasks prior to final dilution[2]. The absorbance of the final solution was measured at 450 mu using a Bausch and Lomb "Spectronic 20" spectrophotometer[3]. The value so obtained was compared to a curve prepared from known standards.

5. Crude fat

Crude fat was determined following extraction with 30 ml petroleum ether (bp 39.5-49.4 C) for 12 hrs on a Goldfish apparatus (AOAC, 1965).

E. STATISTICAL ANALYSIS OF THE DATA

Data in Experiment 1 were analyzed by the analysis of variance with the aid of an IBM 7040 Computer using library program BMD02V, University of Alberta (1965). In Experiment 2, data were analyzed by

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[1]Precision Scientific Co., Chicago, Illinois, U.S.A. (Type A).
[2]Renner, R.O.A. Personal communication, 1968.
[3]Bausch and Lomb Inc., Rochester, N.Y., U.S.A.

the analysis of variance using an IBM 360 Model 67 computer and library program CS017, University of Alberta (1968). Differences between three or more treatment means were determined by Duncan's multiple range test (Steel and Torrie, 1960).

EXPERIMENT 1

A. INTRODUCTION

Consistency of carcass fat is a factor in pork quality. Barber et al. (1957) and Bellis (1961) reported that copper supplementation of the diet adversely affected the carcass grades of market pigs. Taylor and Thomke (1964) observed that dietary copper resulted in a softening of the depot fat as indicated by an increased ($P < .01$) iodine value (Hanus) and a decreased ($P < .01$) consistency value (mm Hg). However, Braude (1965) reported that an attempt to confirm this observation was unsuccessful.

Recent experiments at The University of Alberta confirm the report of Taylor and Thomke (1964). When copper (as $CuSO_4 \cdot 5H_2O$ to supply approximately 250 ppm Cu) was added to barley-fishmeal rations fed to 40 market pigs, 80% of these pigs had soft fat noted on their grading reports. Of these 32 pigs, 52% were graded down for this reason alone. Only 5% of pigs fed the same formulation without a copper supplement had soft fat. When copper was added to barley-soybean meal diets, 19% of the pigs were reported to have soft fat. No soft fat was reported among pigs fed the unsupplemented soybean meal diets (Bowland and Castell, 1964, 1965).

Experiment 1 was designed to obtain further information concerning the effects of dietary copper supplementation on the composition of the depot fat of market pigs.

B. EXPERIMENTAL

Six groups of 4,3-week-old barrows with an average initial

weight of 5.0 kg, assigned on the basis of litter origin and weight, were placed in pens 1.83 × 4.42 m in size. Three groups were fed a basal diet and the other 3 groups the basal diet plus copper. The composition of the diets is given in Table 1. The diets were individually fed, throughout the experimental period, to a scale based on liveweight (Table 2).

A pig from each pen, selected at random at the beginning of the experiment, was killed at average liveweights of 26, 47, 70 and 90 kg. The 26 and 47 kg pigs were sacrificed by stunning and severing the jugular vein. The 70 and 90 kg pigs were marketed through normal commercial channels.

One pig from a copper-supplemented group, that was to be killed at 70 kg, became crippled and was removed from the experiment. For statistical analyses the missing values used were the average of the other 70 kg pigs in the treatment.

Samples of the backfat and perinephric fat were obtained at the time of slaughter as described under General Experimental section B-1. When the samples were prepared for analysis the backfat was separated into its outer and inner layers and a thin slice was taken from the entire length of each layer. The samples of outer and inner backfat and the perinephric fat were chopped into small pieces, and extracted as described under General Experimental section C-1a. Subsequently, the samples were prepared for analysis, analyzed and results calculated as described under General Experimental sections C-1b to d inclusive.

A melting point determination was performed on each sample of outer backfat, inner backfat and perinephric fat obtained in this

TABLE 1

COMPOSITION OF DIETS FOR EXPERIMENT 1

Ingredient	Basal %	Basal plus copper %
Barley, ground	90.10	90.10
Fishmeal, 72%	7.50	7.50
Ground limestone	0.50	0.50
Dicalcium phosphate	1.00	1.00
Salt, iodized	0.50	0.50
Zinc sulfate	0.05	0.05
Vitamin B mix[1]	0.25	0.25
Vitamin B_{12}(19.8 mg/kg)	0.10	0.10
Vitamin A[2]	+	+
Vitamin D[3]	+	+
$CuSO_4 \cdot 5H_2O$[4]	-	+
Composition by analysis		
Crude protein, %	17.7	17.7
Gross energy, kcal/g	3.86	3.86
D.E., kcal/g	3.26	3.26
Copper, ppm	6.1	286.0

[1]Contained the following B vitamins/kg of vitamin mix: riboflavin, 4.4 g; calcium pantothenate, 8.8 g; niacin, 19.8 g; choline chloride, 22.0 g; folic acid, 132.0 mg.

[2]To supply 220,000 IU of vitamin A/100 kg of diet.

[3]To supply 66,000 IU of vitamin D_2/100 kg of diet.

[4]As 0.1% $CuSO_4 \cdot 5H_2O$.

TABLE 2

FEEDING SCALE FOR EXPERIMENT 1[1]

Liveweight range kg	Feed kg/day
5.4 - 9.0	0.45
9.1 - 13.5	0.68
13.6 - 18.1	0.91
18.2 - 22.6	1.09
22.7 - 27.1	1.27
27.2 - 31.7	1.45
31.8 - 36.2	1.59
36.3 - 40.8	1.73
40.9 - 45.3	1.86
45.4 - 49.8	2.00
49.9 - 54.4	2.13
54.5 - 58.9	2.27
59.0 - 63.5	2.36
63.6 - 68.0	2.45
68.1 - 72.5	2.54
72.6 - 77.1	2.63
77.2 - market	2.72

[1]Castell, A.G. 1967. Ph.D. Thesis, University of Alberta.

experiment. A correlation coefficient was calculated for melting point versus fatty acid composition (sum of the unsaturated fatty acids 16:1, 18:1 and 18:2) to ascertain if the more meaningful expression, fatty acid composition, could be used as a reliable indicator of fat softness in place of melting point.

C. RESULTS AND DISCUSSION

To facilitate presentation of the data the sum of the saturated acids (14:0, 16:0 and 18:0) and the sum of the unsaturated acids (16:1 + 18:1 + 18:2) was obtained. Together these represented greater than 95 percent of the fatty acids. The results of this summation are shown in Fig. 2, 3 and 4 for outer backfat, inner backfat and perinephric fat, respectively. In both the outer and inner backfat layers there was a lower ($P < .01$) percentage of the saturated acids amongst pigs receiving the copper supplemented ration as compared to pigs receiving the basal ration at 26, 47 and 70 kg liveweight. At 90 kg liveweight the apparent differences were not significant. A similar trend was observed in the perinephric fat. Copper supplementation resulted in a decreased ($P < .05$) percentage of the saturated acids at 26 kg liveweight. At 47 and 70 kg liveweight the same differences occurred (significant at the $P < .01$ level) while at 90 kg the apparent differences were not significant.

Data for the individual fatty acids are presented in Table 3. Generally the increased percentage of unsaturated fatty acids in pigs fed copper supplemented diets could be accounted for by significantly greater amounts of 16:1 and 18:1. Similarly the decreases in saturated fatty acids could be accounted for by significantly lesser

amounts of 16:0 and 18:0.

The average melting points of depot fat samples from the pigs fed either the basal or the copper supplemented diet were 31.5°C and 25.2°C, respectively while the average weight % of the unsaturated fatty acids (16:1 + 18:1 + 18:2) in these samples were 56.5 and 65.3, respectively. Melting point is the expression commonly used to indicate softness of a fat sample. In the present and subsequent experiments, we wished to express softness of porcine depot fats in terms of the percentage of unsaturated fatty acids (16:1 + 18:1 + 18:2) present in the fat. To determine if this was a true measure of softness, the correlation of melting point with the percent unsaturated fatty acids was calculated using the data for both these parameters obtained by the analyses of the outer and inner backfat and the perinephric fat samples. A negative ($P < .01$) correlation existed between the melting point of a sample and the percent unsaturated fatty acids in the sample.

The significant ($P < .01$) correlation coefficient, ($r = -.814$) indicated that as melting point of a sample decreased the percentage of unsaturated fatty acids in the sample increased and that 64.4% ($r^2 \times 100$) of the difference in mp could be explained by differences in the sum of the unsaturated fatty acids in the sample. The regression coefficient ($b = -.870$) indicated that for each decrease of .87°C in melting point, one could expect a one percent increase in percentage of total unsaturated fatty acids present in the sample. Thus, percentage of unsaturated fatty acids can be used as a reliable indicator of melting point and hence of fat softness in these studies.

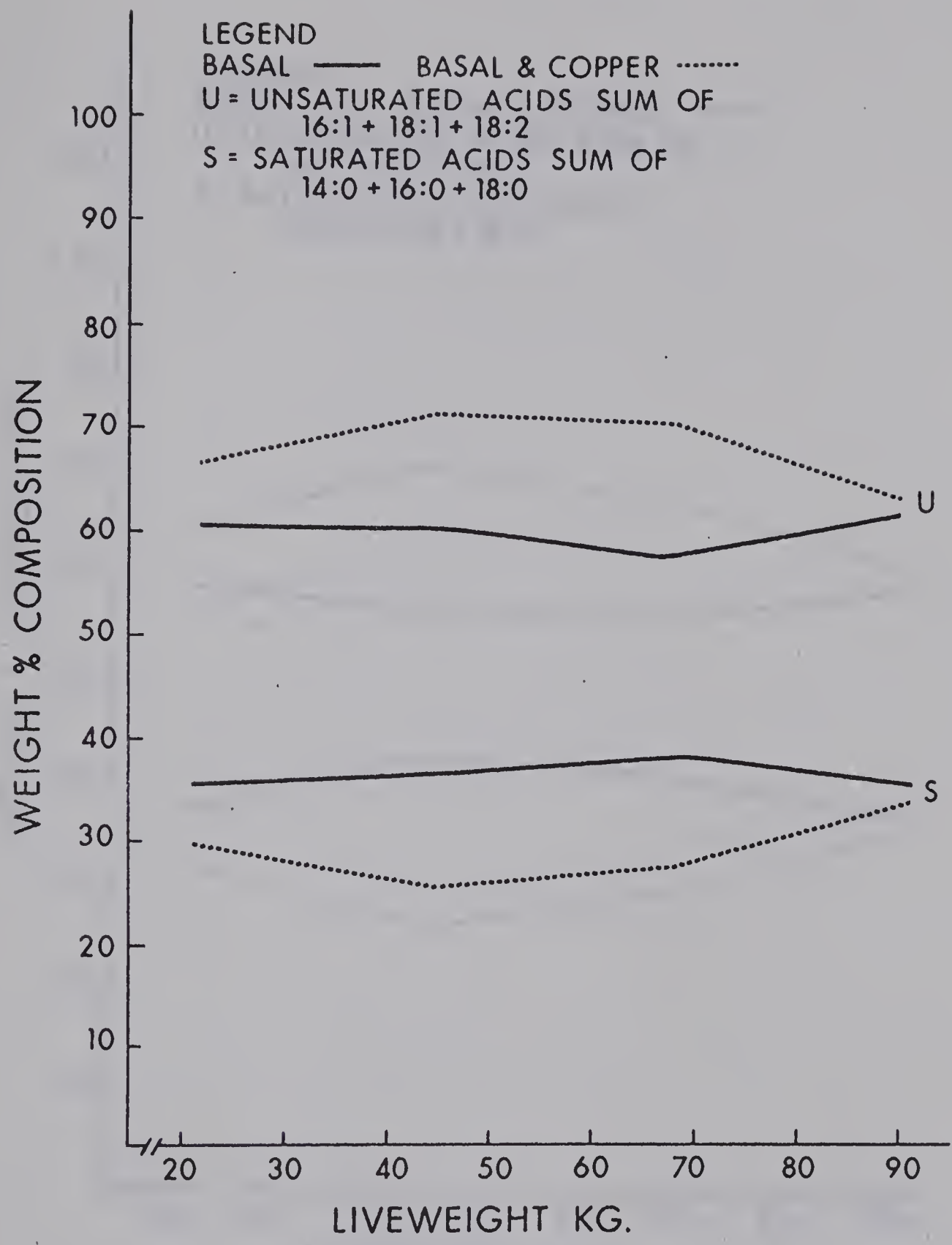

Figure 2. A comparison of levels of saturated and unsaturated fatty acids present in the outer backfat layer of pigs fed barley-fishmeal diets, with or without supplemental copper, to a scale based on liveweight.

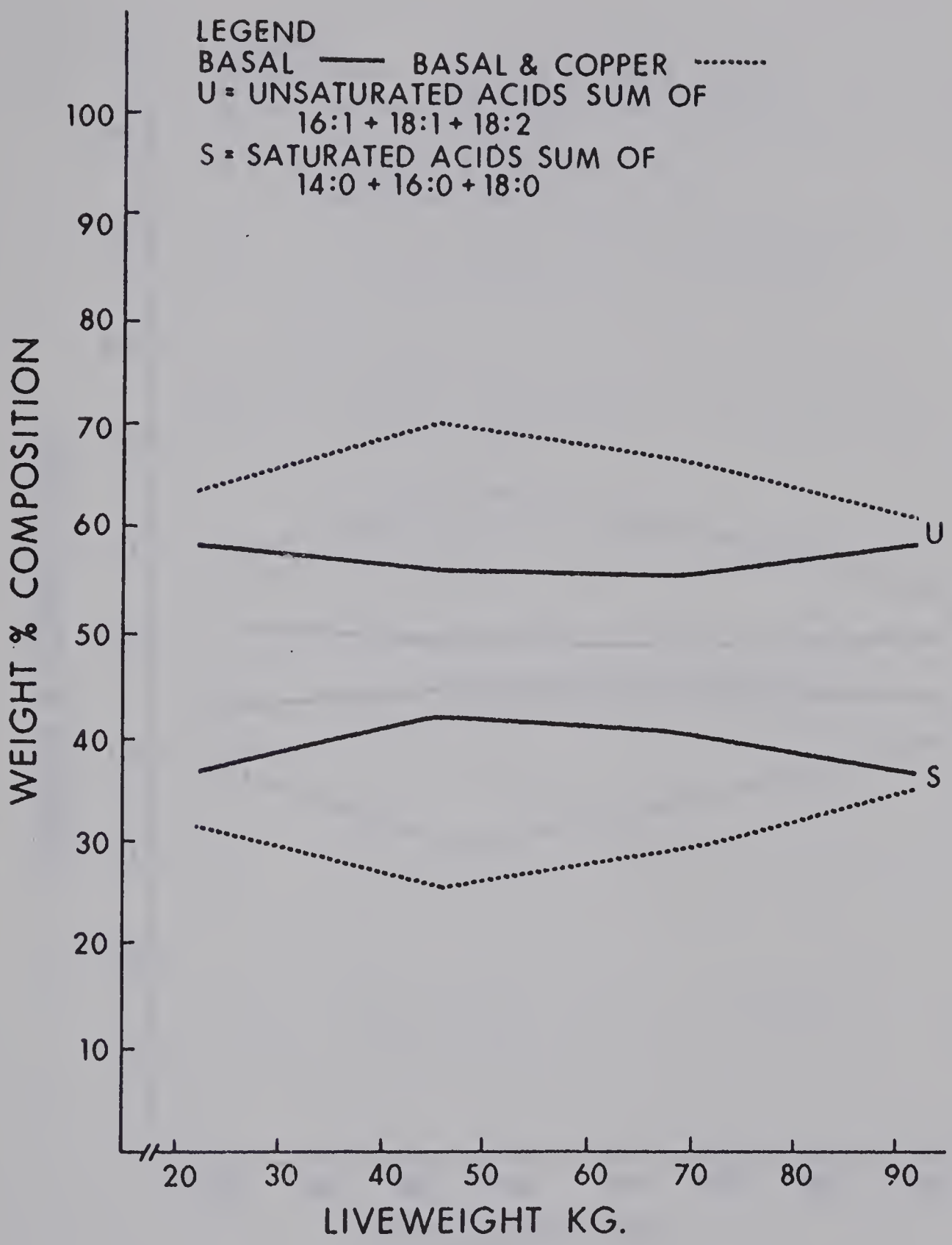

Figure 3. A comparison of levels of saturated and unsaturated fatty acids present in the inner backfat layer of pigs fed barley-fishmeal diets, with or without supplemental copper, to a scale based on liveweight.

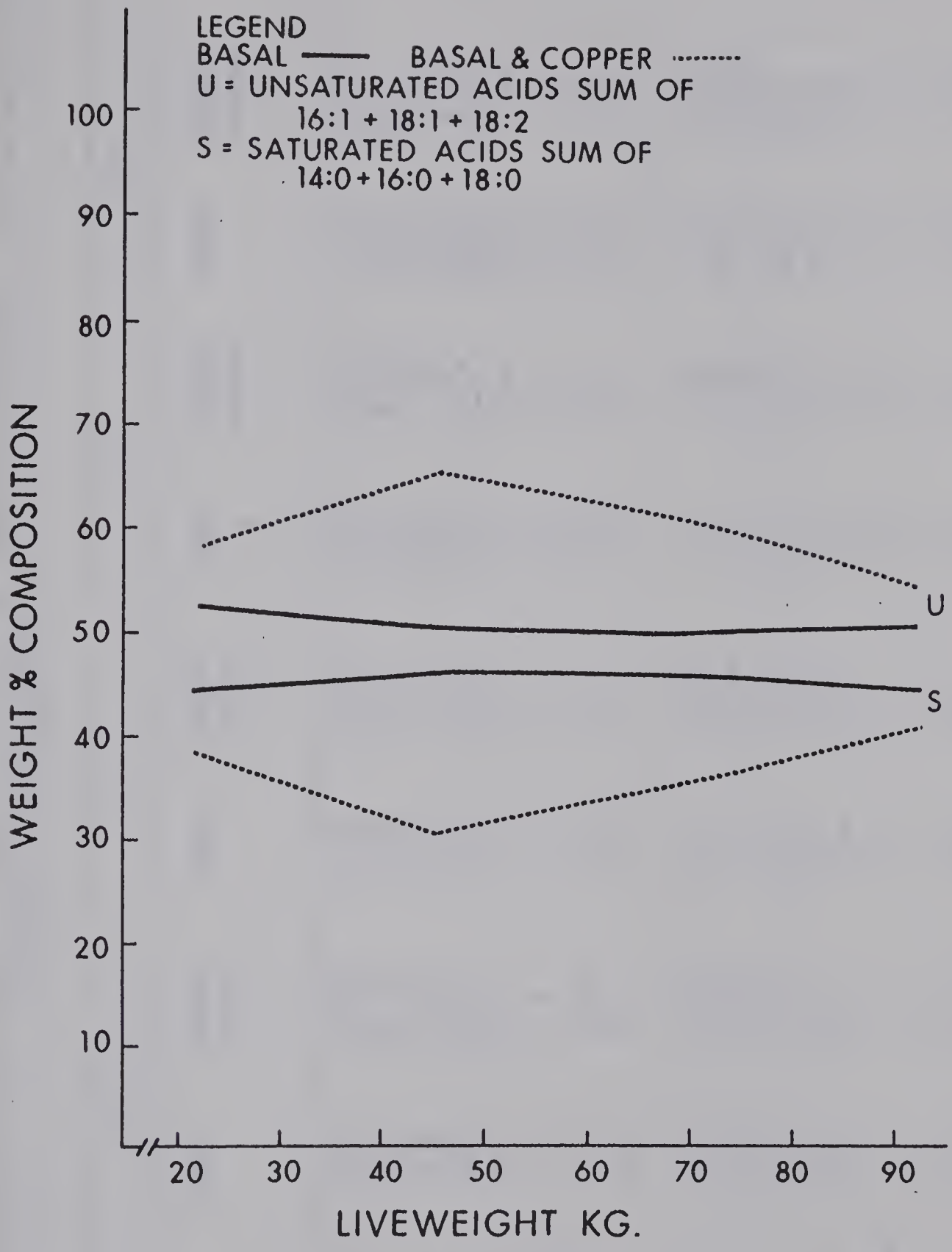

Figure 4. A comparison of levels of saturated and unsaturated fatty acids present in the perinephric fat of pigs fed barley-fishmeal diets, with or without supplemental copper, to a scale based on liveweight.

TABLE 3

PERCENT FATTY ACID COMPOSITION OF DEPOT FATS OF PIGS FED A BASAL OR COPPER SUPPLEMENTED DIET AND SACRIFICED AT SEVERAL LIVEWEIGHTS[1 & 2]

Liveweight kg.		26		47		70		90	
Area Sampled	Diet / Fatty acid	Basal	Basal+ copper	Basal	Basal+ copper	Basal	Basal+ copper	Basal	Basal+ copper
Outer backfat	14:0	1.8	2.0[a]	1.6	2.1[a]	1.6	1.6[b]	1.5	1.7[a]
	16:0	24.3	21.2[b]	24.0	18.4[b]	24.2	18.4[b]	23.3	22.9
	16:1	4.4	6.2[a]	4.0	9.9[a]	3.4	7.6[a]	3.6	5.5[b]
	18:0	9.8	6.1[b]	11.0	5.2[b]	12.4	7.3[b]	10.9	8.8
	18:1	46.0	48.8[a]	47.3	49.9[a]	46.6	54.4[a]	49.8	50.5[b]
	18:2	10.6	12.0	9.3	11.6[a]	7.8	8.4	8.3	7.6[b]
	18:3 + 20:0	1.2	1.2	1.1	1.1	0.9	0.8	0.9	0.9
	1^3_3	1.6	2.0[a]	1.5	1.7[a]	1.6	1.4	1.5	1.8
	2^3	0.3	0.3	0.2	0.2	1.5	0.1	0.2	0.2
Inner backfat	14:0	1.7	1.9[a]	1.4	2.0[a]	1.8	1.6[b]	1.4	1.6[a]
	16:0	24.9	21.8[b]	25.3	18.3[b]	25.5	19.2[b]	24.3	23.5[a]
	16:1	3.8	5.6[a]	3.3	9.8[a]	3.1	6.8[a]	3.3	5.0[a]
	18:0	11.2	7.8[b]	13.6	5.7[b]	13.9	8.7[b]	12.0	10.1
	18:1	45.0	48.0	45.4	50.1[a]	46.1	54.0[a]	48.9	50.4
	18:2	10.3	11.3	8.4	11.1[a]	6.9	7.1	7.5	6.6
	18:3 + 20:0	1.1	1.1	0.9	1.0	0.9	0.8	0.8	0.7
	1^3_3	1.7	2.1	1.6	1.8	1.7	1.6	1.6	1.7
	2^3	0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.3

..... cont'd

TABLE 3. continued

Liveweight kg.		26		47		70		90	
Area Sampled	Diet / Fatty acid	Basal	Basal+ copper	Basal	Basal+ copper	Basal	Basal+ copper	Basal	Basal+ copper
Perinephric fat	14:0	1.7	2.0[a]	1.7	1.8	1.6	1.4[b]	1.4	1.5
	16:0	26.7	24.5	27.4	20.3[b]	28.6	21.6[b]	27.6	25.2
	16:1	4.1	5.5[a]	3.6	7.9[a]	3.4	4.8[a]	3.2	4.2
	18:0	16.0	11.1[b]	17.3	8.8[b]	16.6	12.2[b]	16.7	14.3
	18:1	36.8	40.0	38.0	46.2	39.2	49.2[a]	40.7	44.4
	18:2	11.4	13.1	9.0	11.7[a]	8.0	7.9	7.6	7.6
	18:3 + 20:0	1.5	1.5	1.3	1.4	1.2	1.0	1.2	1.1
	1^3_3	1.6	1.9	1.5	1.7	1.3	1.7[a]	1.4	1.7[a]
	2^3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2

1. Each value is the average of duplicate analysis on 3 pigs.
2. Superscript "a" indicates significantly ($P < .01$ or $P < .05$) greater amount of the acid in question in copper supplemented pigs. Similarly superscript "b" indicates significantly ($P < .01$ or $P < .05$) lesser amounts. Absence of superscript indicates no significant difference.
3. Unidentified peaks.

There are three possible means by which a depot fat can be softened: by an increase in the proportion of unsaturated fatty acids present; by an increase in the proportion of short chain fatty acids present; or by changes in the structure of the component triglycerides. The data presented (Table 3) indicate that copper supplementation of the diet softens the depot fats of pigs by increasing the proportion of unsaturated fatty acids, notably 16:1 and 18:1, present therein. There is also an indication that a slight increase in the proportion of a shorter chain fatty acid, 14:0, was involved (Table 3), at least in the case of the outer and inner backfat layers. Previous experiments at The University of Alberta (Bowland and Castell, 1964, 1965) support the observation that copper supplementation softens depot fat in pigs but specific changes in fat composition responsible for the softening were not determined.

It has been suggested, Taylor and Thomke (1964) that high levels of dietary copper could: influence the absorption of dietary fat components; influence the mobilization from or deposition of fatty acids in the depot fat; or that copper stored in the liver might interfere with endogenous fat metabolism. The present experiment does not reveal the site of action of copper in fat metabolism. An increase in linoleic acid (18:2) in the depot fat of copper supplemented pigs was only observed in pigs 47 kg in weight. Linoleic acid is an essential fatty acid not synthesized by the pig. If copper had exerted its effect by causing preferential absorption of unsaturated fatty acids present in the diet, then one would expect that the increase in unsaturated fatty acids in the adipose tissue, caused by copper supplementation of the diet, would have been consistently

contributed to by linoleic acid. The data suggest, therefore, that the site of action of copper is systemic rather than enteric.

In the present experiment, there was not a significantly higher proportion of unsaturated fatty acids at 90 kg (market weight) among the copper supplemented pigs than among the control pigs nor was soft fat noted on the grading reports. These pigs had been fed to scale and averaged 202 days of age at the time of marketing. In the previous experiments at The University of Alberta in which 80 percent of the pigs were reported to have had soft fat, the pigs had been fed the same diet ad libitum and averaged 155 days of age at the time of marketing. In the present experiment, there was a significantly greater proportion of the unsaturated fatty acids in the depot fats of copper supplemented pigs when they weighed 70 kg and average 168 days of age. This observation suggests two points. Firstly, the effect of copper on the degree of unsaturation of porcine depot fat appears to be related to feeding system and/or age. Secondly, the failure of the British workers, Braude (1965), to confirm the results of Taylor and Thomke (1964), may be due to the fact that in Britain, market pigs are generally fed to scale, as in the present experiment, and are marketed at an older age while the pigs used by Taylor and Thomke (1964) were fed ad libitum.

In our earlier experiments, the greatest incidence of soft fat was among pigs fed a copper supplemented diet in which fishmeal was the protein supplement while pigs fed a diet containing copper and soybean meal exhibited fat softness to a lesser degree (Castell and Bowland, 1968a). Possibly, there may be an interaction between copper and protein source which results in soft depot fats. This

possibility is supported by the observations of Barber et al. (1962) who found that copper supplementation of the diet had a greater effect on ADG and FC when added to diets containing FM than when added to diets containing SM as the protein supplement. Taylor and Thomke (1964) did not indicate the composition of the diet fed.

D. SUMMARY

Twenty-four Hampshire × Yorkshire barrows were fed to scale a diet in which fishmeal was the protein supplement with and without copper. Copper supplementation resulted in a significant increase in the proportion of unsaturated fatty acids in the outer backfat, inner backfat and perinephric fat at 26, 47 and 70 kg liveweight. There was a corresponding decrease in the proportion of saturated fatty acids. The increase in the proportion of unsaturated fatty acids was associated with a decrease in the melting point of the depot fats. No significant differences were found in fatty acid composition of these fats at 90 kg liveweight, although the former pattern of distribution still existed. The increase in the proportion of unsaturated acids could be accounted for largely by increases in 16:1 and 18:1 with corresponding decreases in 16:0 and 18:0.

EXPERIMENT 2

A. INTRODUCTION

In previous experiments at The University of Alberta, (Bowland and Castell, 1964, 1965) the incidence of soft fat in the carcasses of pigs fed barley-soybean meal diets supplemented with copper was lower than the incidence in carcasses from pigs fed barley-fishmeal diets supplemented with copper (19% versus 52%, respectively). In addition, Barber et al. (1962) and Castell and Bowland (1968a) observed that copper supplementation of the diet had a greater positive effect on ADG and FC when added to diets containing fishmeal than when added to diets containing soybean meal as the protein supplement. The results of Experiment 1 confirmed the effect of dietary copper supplementation on depot fat composition in a barley-fishmeal diet but did not provide any information on possible effects if other protein supplements were used. Experiment 2 was designed to study the effect of copper supplementation, in diets containing either fishmeal, meat meal, soybean meal or rapeseed meal as the sole source of supplemental protein, on the fatty acid composition of porcine depot fat. The protein sources chosen were those most likely to be incorporated into practical swine diets in Alberta.

As indicated in the discussion following Experiment 1, there appears to be a relationship between age at market and/or feeding system (restricted versus ad libitum) and the fat softening effect of supplemental copper. Soft fat among pigs fed diets containing supplemental copper was first noted with pigs fed ad libitum. The pigs in Experiment 1 were fed to a scale based on liveweight and did not have

soft fat at market. Feeding to scale is a system widely used in Europe but not in North America. Therefore, the pigs in Experiment 2 were fed ad libitum, the feeding system commonly used in North America and the system most likely to produce soft fat when copper supplemented diets are fed.

B. EXPERIMENTAL

Sixty-four pigs equalized as to sex and randomized with respect to litter were allotted in October, 1967 at an average weight of 9.5 kg and an average age of 40 days to eight dietary groups (Table 4). The pigs were managed as described under General Experimental, Section D, and were housed in a barn designed for individual feeding. The pens measured 0.62 m wide × 1.23 m long and had a half-slotted concrete floor. The pigs were hand-fed their assigned diet so as to provide ad libitum feed intake. The composition of the diets used is given in Table 5. The four protein sources were added to the diets at levels calculated to produce isonitrogenous diets. The same diets were fed from weaning to market.

The pigs were weighed at weekly intervals and weekly feed consumption was recorded. Backfat samples were obtained by needle biopsy, as previously described (General Experimental B-1), from each pig at average liveweights of 34.4, 45.4, 57.0, 68.0 and 78.9 kg. In addition to the biopsy samples, samples of the outer and inner backfat layers and the perinephric fat were obtained from each pig at slaughter (average weight 88.4 kg). The outer backfat samples obtained at slaughter were taken from the same site as the biopsy samples. All samples were handled and analyzed as previously described

(General Experimental C-1 (a-d)).

TABLE 4

ALLOTMENT OF PIGS IN EXPERIMENT 2

Dietary group	No. of pigs	Protein source	Supplemental copper
1	8	barley-fishmeal	0
2	8	barley-fishmeal	+
3	8	barley-meat meal	0
4	8	barley-meat meal	+
5	8	barley-soybean meal	0
6	8	barley-soybean meal	+
7	8	barley-rapeseed meal	0
8	8	barley-rapeseed meal	+

Apparent digestibility of nitrogen and energy was determined at approximately 34 and 68 kg, using one barrow and one gilt from each dietary group; the chromic oxide indicator method being employed. Sixty-eight kg of each diet was set aside and to this was added 0.5% (340 g) Cr_2O_3[1] and 0.5% (340 g) corn oil to aid in the distribution of the indicator. The diets were then mixed thoroughly with the aid of a Samson Mixall[2] cone mixer and a representative sample of the feed

[1]Fisher Scientific Co., Fairlawn, New Jersey. Certified reagent grade.
[2]Chillicothe Industries Inc., Kansas City, Missouri.

TABLE 5

COMPOSITION OF DIETS AND DIGESTIBILITY OF ENERGY AND NITROGEN IN EXPERIMENT 2

Protein source		Barley-fishmeal		Barley-meat meal		Barley-soybean meal		Barley-rapeseed meal	
Dietary group		1	2	3	4	5	6	7	8
Ingredient, %									
Barley		90.0	90.0	87.6	87.6	82.0	82.0	77.0	77.0
Fishmeal		7.6	7.6	-	-	-	-	-	-
Soybean meal		-	-	-	-	15.0	15.0	-	-
Meat meal		-	-	11.5	11.5	-	-	-	-
Rapeseed meal		-	-	-	-	-	-	20.0	20.0
Ground limestone		0.5	0.5	-	-	1.0	1.0	1.0	1.0
Dicalcium phosphate		1.0	1.0	-	-	1.1	1.1	1.1	1.1
Iodized salt		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Zinc sulfate		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin B mix[1]		0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin B_{12}[2]		0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin A[3]		+	+	+	+	+	+	+	+
Vitamin D_2[4]		+	+	+	+	+	+	+	+
$CuSO_4 \cdot 5H_2O$		0	+	0	+	0	+	0	+
Compostion									
By analysis									
Crude protein, %		13.8		14.1		14.2		14.6	
Gross energy, kcal/g		3.87		3.74		3.73		3.79	
Crude fat, %		1.90		2.21		1.20		1.64	
By calculation									
Copper added, ppm		0	254.5	0	254.5	0	254.5	0	2.54.5
Apparent digestibility, % (dry matter basis)									
Nitrogen	34 kg	63.1	60.4	58.3	66.8	66.1	73.5	67.1	69.3
	68 kg	62.2	63.6	61.1	63.0	59.3	66.3	64.9	67.5
Energy	34 kg	68.0	62.5	64.5	69.8	68.1	72.9	66.0	70.2
	68 kg	73.9	70.5	65.6	68.3	67.1	69.9	66.9	69.3

[1] 249-C contains 4.4 g riboflavin; 19.8 g niacin and 8.8 g pantothenic acid, 22.0 gm choline chloride, 132.0 mg folic acid.

[2] 44 mg vitamin B_{12}/kg.

[3] To supply 220,000 IU vitamin A/100 kg.

[4] To supply 66,000 IU vitamin D_2/100 kg.

[5] To supply approximately 250 ppm copper (0.1 kg $CuSO_4 \cdot 5H_2O$/100 kg diet).

was obtained. The animals were changed to the Cr_2O_3 supplemented feed on the weekly weigh-day that their weight approximated 34 or 68 kg. The feed containing Cr_2O_3 was fed for five days before fecal samples were collected. Feces were then collected for two consecutive days; the samples were pooled, prepared and analyzed for energy, nitrogen, dry matter and chromic oxide as previously described (General Experimental, Section D 1-4). Samples of feed and feces were ground, using a Wiley mill[1], to pass through a 40 mesh screen prior to analysis for Cr_2O_3.

During the experimental period several animals became severely crippled possibly due to close confinement and it was necessary to remove them from the experiment. Examination of some of these pigs by the Veterinary Diagnostic Laboratory[2] failed to result in a diagnosis other than severe crippling. Loss of pigs due to crippling was not restricted to any one dietary group. Four pigs were lost from basal groups and seven pigs were lost from copper supplemented groups. No pigs were lost from the group fed the barley-rapeseed meal diet without supplemental copper. The losses cannot be attributed to the addition of copper to the diet. When pigs were removed from the experiment, missing values (average of the other animals in the group) were substituted in the data before statistical analysis. Error degrees of freedom were reduced to account for these missing value substitutions.

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[1]Arthur H. Thomas Co., Philadelphia, P.A., U.S.A.

[2]Government of Alberta, Department of Agriculture, Veterinary Services Division, Field Services Branch, 6909-116 Street, Edmonton, Alberta.

C. RESULTS AND DISCUSSION

1. Growth and carcass characteristics

The data for average daily feed (ADF), ADG, FC and carcass characteristics were analyzed statistically as a 4 × 2 × 2 factorial consisting of four protein supplements, two levels of copper supplementation (0 versus 250 ppm) and two sexes (barrows versus gilts). Since the same diets were fed from weaning to market the data were examined for the overall period only, no attempt being made to subdivide the growth data into starting, growing and finishing periods.

Statistical analysis revealed no significant interactions, thus only main effects attributable to protein source, supplemental copper and sex are discussed. The data for these factors are presented in Table 6.

The protein level of the diets used in this experiment was lower (Table 5) than is recommended for the starting and growing pigs (NAS-NRC, 1964). As a result, the pigs gained more slowly and were less efficient than would normally be expected. However, since all diets contained a similar level of protein this becomes a constant factor and should not affect the observed trends. Low total protein in the diet results in protein quality (amino acid composition) of the protein supplements assuming greater importance.

Source of supplemental protein had no effect on feed intake, dressing percentage, carcass length or R.O.P. score, however, source of protein significantly affected ADG, FC, age to market and total backfat thickness. Pigs fed the barley-fishmeal diet gained faster

TABLE 6

EFFECT OF SOURCE OF SUPPLEMENTAL PROTEIN, COPPER SUPPLEMENTATION AND SEX ON THE GROWTH AND CARCASS CHARACTERISTICS OF MARKET PIGS[1]

	ADF kg	ADG kg	FC kg/kg	Age to market days	Dressing %	Carcass length cm	Total backfat cm	ROP score
Protein source								
Fishmeal	2.5	0.66[a]	3.86[a]	177[a]	79.0	74.2	3.98[a]	76.2
Meat meal	2.4	0.59[c]	4.12[b]	195[b]	78.1	75.0	4.01[a]	76.5
Soybean meal	2.5	0.64[a]	3.94[a]	180[a]	78.7	73.8	3.89[a]	76.5
Rapeseed meal	2.4	0.55[c]	4.32[b]	200[b]	77.9	74.0	3.59	77.9
Copper								
0	2.4	0.61	4.10	188	78.6	74.5	3.88	76.7
+	2.4	0.61	4.10	188	78.3	74.0	3.85	76.8
Sex								
Barrows	2.5*	0.63*	4.10	186	78.1	74.0	4.07*	75.6*
Gilts	2.4	0.59	4.10	190	78.8	74.5	3.66	77.9

[1]Means followed by the same superscript are not significantly ($P < .05$) different.

*Significant ($P < .05$).

than did pigs fed barley-soybean meal who in turn gained faster than those fed the barley-meat meal or barley-rapeseed meal diets. There were no significant differences noted in ADG between pigs fed the barley-meat meal or barley-rapeseed meal diets. Pigs receiving either the barley-fishmeal or the barley-soybean meal diets were equally efficient at converting feed to gain and were more efficient than those receiving either the barley-meat meal or barley-rapeseed meal diets. Pigs fed the barley-fishmeal diet and the barley-soybean meal diet reached market weight at a younger chronological age than did those fed either the barley-meat meal or barley-rapeseed meal diets. Pigs receiving the barley-rapeseed meal diet had less total backfat than pigs in the other three dietary groups among which no significant differences were noted with respect to this parameter.

The differences in performance noted in the foregoing discussion may be attributed to differences in the protein quality of the diets. Morgan and Robinson (1962) note that lysine is likely to be the first-limiting amino acid in pig diets. Lysine, as a percent of crude protein, calculated on an as-fed basis was 5.7%, 5.4%, 4.5% and 4.8% for the barley-fishmeal, barley-soybean meal, barley-meat meal and barley-rapeseed meal diets, respectively. The lysine levels of these diets were 0.78, 0.76, 0.63 and 0.70%, respectively as compared to a recommended level (NAS-NRC, 1964) of 1.40% and 0.75% for the starting and growing pig, respectively. The lower levels of lysine in the barley-meat meal and barley-rapeseed meal diets may have reduced gain, adversely affected feed conversion and concomitantly increased the time required for the animals to attain market weight. Zimmerman, Peo and Hudman (1967) have reported that when 10% meat and bone scrap replaced

soybean meal in a corn-soybean meal diet a significant reduction (0.04 kg) in ADG was observed. Wilson and Holder (1967) reported that pigs fed wheat-fishmeal diets gained faster than pigs fed wheat-meat and bone meal diets. The level of rapeseed meal used in these diets (20%) is approximately double the recommended level. Bowland (1966) states that: "For market pigs, 25 to 90 kg liveweight, rapeseed meal may be used as up to 10% of the total ration. Feed intake and rate of gain may be reduced at this level of feeding but efficiency of feed utilization is not affected." In the present experiment, feed conversion was also adversely affected, possibly due to the poor amino acid balance in the barley-rapeseed meal diet.

Supplemental copper did not significantly affect any of the growth or carcass characteristics studied. These results are contrary to the majority of published reports on the effects of dietary copper supplementation, however, are in general agreement with the results of previous experiments at The University of Alberta for the overall period from weaning to market (Castell and Bowland, 1968a). The failure of supplemental copper to result in improvements in ADG and FC cannot, necessarily be attributed to the low dietary protein level although protein might be a factor. Previously discussed results have indicated that the effect of protein level on the response to copper supplementation is subject to considerable variation.

Sex (barrows versus gilts) significantly affected performance and certain carcass characteristics. Barrows consumed more feed per day and gained faster than did the gilts; both sexes were equally efficient as converters of feed to gain. No significant differences

were found between sexes with respect to dressing %, carcass length or age to market; but barrows had a significantly greater total backfat thickness and a concomitantly lower R.O.P. score than gilts.

2. Fatty acid composition of the depot fat

(a) Outer backfat

To facilitate presentation of the data, the sum of the unsaturated fatty acids (16:1 + 18:1 + 18:2) and the sum of the saturated fatty acids (14:0 + 16:0 + 18:0) were obtained for the outer backfat (OBF). The data in this form was analyzed by analysis of variance as a 4 × 2 × 2 × 6 factorial involving four sources of supplemental protein, (fishmeal, meat meal, soybean meal and rapeseed meal),two levels of supplemental copper (0 or 250 ppm), two sexes (barrows and gilts) and six liveweights (34, 45, 57, 68, 79 and 88 kg). Only main effects and two factor interactions are discussed as three factor interactions are of doubtful biological or practical significance. The analysis of variance of the data is presented in the Appendix, Table 1.

In addition to the data for the OBF the sum of the unsaturated and saturated fatty acids was obtained from the analysis of samples of the inner backfat (IBF) and perinephric fat (PF) at 88 kg. These data were not subjected to analysis of variance.

The sums of the saturated and unsaturated fatty acids together represented greater than 95% of the fatty acids. As established in Experiment 1, the sum of the unsaturated fatty acids (or conversely the sum of the saturated fatty acids) can be used as a reliable indicator of fat softness.

The levels of the saturated and unsaturated fatty acids were influenced ($P < .05$) by source of supplemental protein, level of copper supplementation, sex and liveweight at sampling. Means for these factors and their two factor interactions are presented in Table 7.

1. Effects of source of supplemental protein

The OBF of pigs receiving each of the four diets differed significantly in levels of unsaturated fatty acids (UFA). In decreasing levels of UFA the diets ranked barley-fishmeal, barley-meat meal, barley-soybean meal and barley-rapeseed meal. The trend was the opposite of this for the SFA except that no significant difference existed between the barley-soybean meal and the barley-rapeseed meal diets.

The effects of source of supplemental protein on the levels of UFA and SFA is difficult to explain. The levels of crude fat in the diets (Table 5) would not be expected to exert a significant effect on the fatty acid composition of the depot fat, the sum of UFA and SFA being a reflection of this compostion. Hilditch and Williams (1964) quote the work of Ellis, Rothwell and Pool (1931b) in which pigs were fed a basal diet containing less than 1% fat, supplemented by varying amounts of cottonseed oil, an oil relatively high in linoleic acid. The results showed that between four and eight % of added fat was required before the fatty acid composition of the backfat was significantly altered.

2. Effects of copper supplementation of the diet

Copper supplementation of the diet increased ($P < .01$) the

TABLE 7

PROPORTIONS OF UNSATURATED AND SATURATED FATTY ACIDS IN THE OUTER BACKFAT LAYER OF PIGS AS AFFECTED BY TYPE OF PROTEIN SUPPLEMENT, LEVEL OF COPPER SUPPLEMENTATION, SEX AND WEIGHT OF PIG

Main effects				Interactions			
		Sum of 16:1, 18:1 18:2 %	Sum of 14:0, 16:0 18:0			Sum of 16:1,18:1 18:2 %	Sum of 14:0,16:0 18:0
1. Protein	FM	68.6[a]	28.8[a]	1. Protein x copper	FM 0	65.3	32.1
	SM	63.5[c]	33.9[b]		FM +	71.9	25.5
	MM	65.8[b]	31.5[c]		SM 0	61.8	35.6
	RM	62.2[d]	33.8[b]		SM +	65.2	32.2
					MM 0	63.8	33.8
2. Copper	0	63.2**	33.9**		MM +	67.9	29.2
	+	66.9	30.1		RM 0	61.8	34.1
					RM +	62.6	33.5
3. Sex	M	64.3**	32.9**				
	F	65.7	31.1	2. Protein x sex	FM-M	68.5	28.9
					FM-F	68.6	28.7
4. Weight	34	66.6[cd]	30.5[a]		SM-M	62.0	35.6
	45	65.8[c]	31.0[ab]		SM-F	65.0	32.2
	57	64.7[b]	32.4[c]		MM-M	64.9	32.5
	68	66.3[cd]	30.7[ab]		MM-F	66.8	30.5
	79	63.9[ab]	33.3[cd]		RM-M	61.8	34.5
	88	62.9[a]	34.0[d]		RM-F	62.5	33.1
				3. Copper x sex	M 0	62.9	34.4
					M +	65.7	31.4
					F 0	63.5	33.4
					F +	68.0	28.8
				4. Copper x weight	34 0	64.8	32.3
					+	68.4	28.7
					45 0	63.9	33.1
					+	67.7	29.0
					57 0	62.2	34.6
					+	67.1	30.1
					68 0	64.3	32.8
					+	68.2	28.6
					79 0	63.2	34.0
					+	64.6	32.5
					88 0	60.7	36.4
					+	65.1	31.6

[1]Means followed by the same superscript are not significantly different ($P < .05$).

**Significant ($P < .01$).

proportion of UFA and decreased the proportion of SFA in the OBF of pigs as compared to the levels present in the OBF of those receiving the unsupplemented basal diets. As the objective of this experiment was to study the effect of copper supplementation, in diets containing several protein supplements, on the fatty acid composition of porcine depot fat, the data has been further subdivided. The sum of the UFA and SFA obtained from analysis of the OBF, at six liveweights, of pigs fed either a basal or a copper supplemented diet are presented in Figs. 5, 6, 7 and 8 for the barley-fishmeal, barley-meat meal, barley-soybean meal and barley-rapeseed meal diets, respectively. In addition, the data for the weight % of the individual fatty acids at each liveweight and for the overall period are presented in Table 8 for each source of supplemental protein.

Copper supplementation of the barley-fishmeal diet resulted in higher levels of UFA and lower levels of SFA in the OBF at all weights in comparison with levels present in the OBF of pigs fed the unsupplemented basal diet (Fig. 5). The OBF of the copper supplemented pigs contained greater amounts of 18:2 and 16:1 and lesser amounts of 16:0 and 18:0 at all weights and for the overall period. In Experiment 1 levels of 18:2 although tending to increase due to dietary copper supplementation only significantly increased at 47 kg. The levels of 18:1 in Experiment 2 were only significantly higher in the overall period, in contrast to the results of Experiment 1 (Table 3) in which levels of 18:1 were significantly increased by copper supplementation of the diet at 26, 47 and 70 kg. In addition, there tended to be a higher level of 14:0 in the OBF of copper

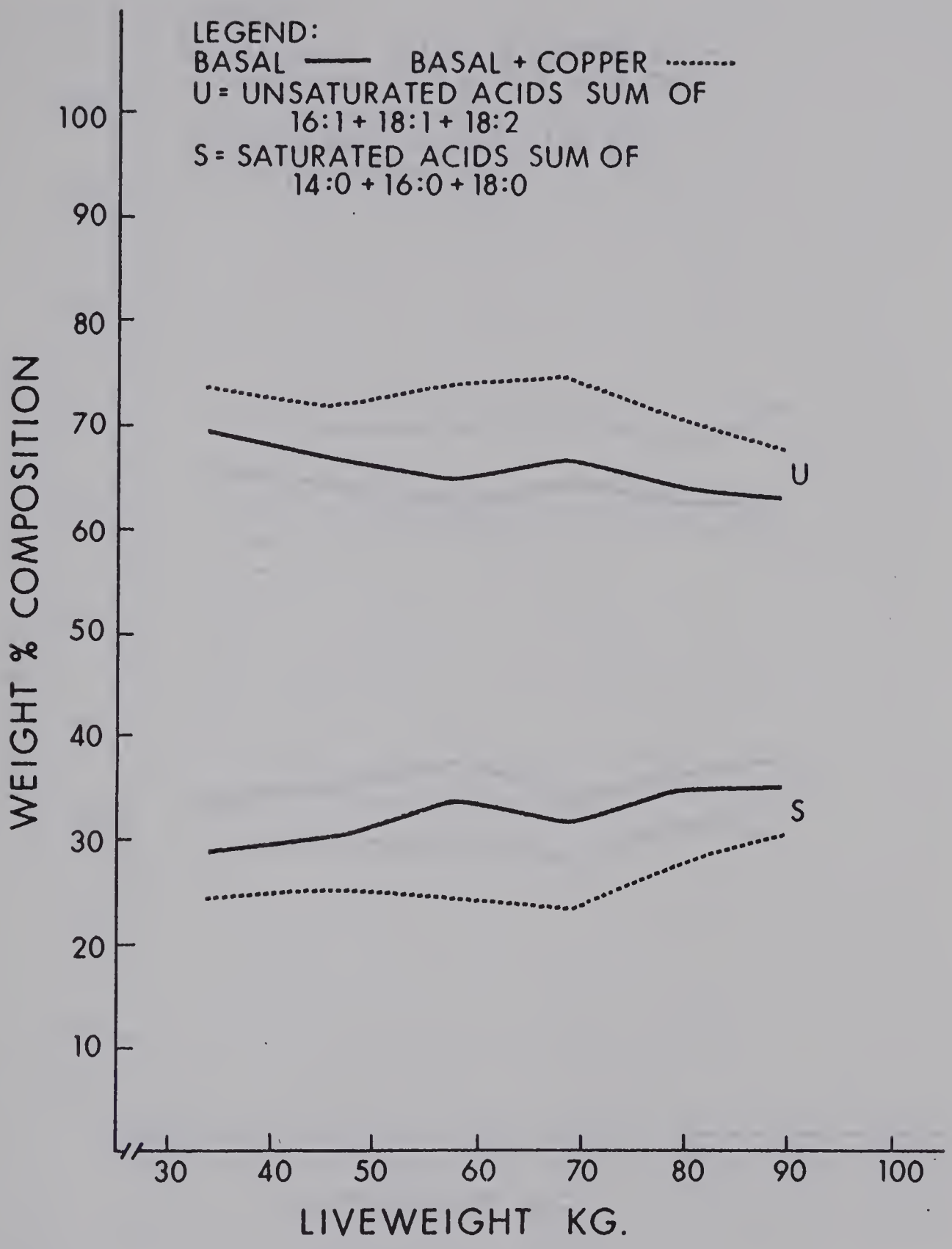


Figure 5. A comparison of levels of saturated and unsaturated fatty acids present in the backfat of pigs fed barley-fishmeal diets, with or without supplemental copper, ad libitum.

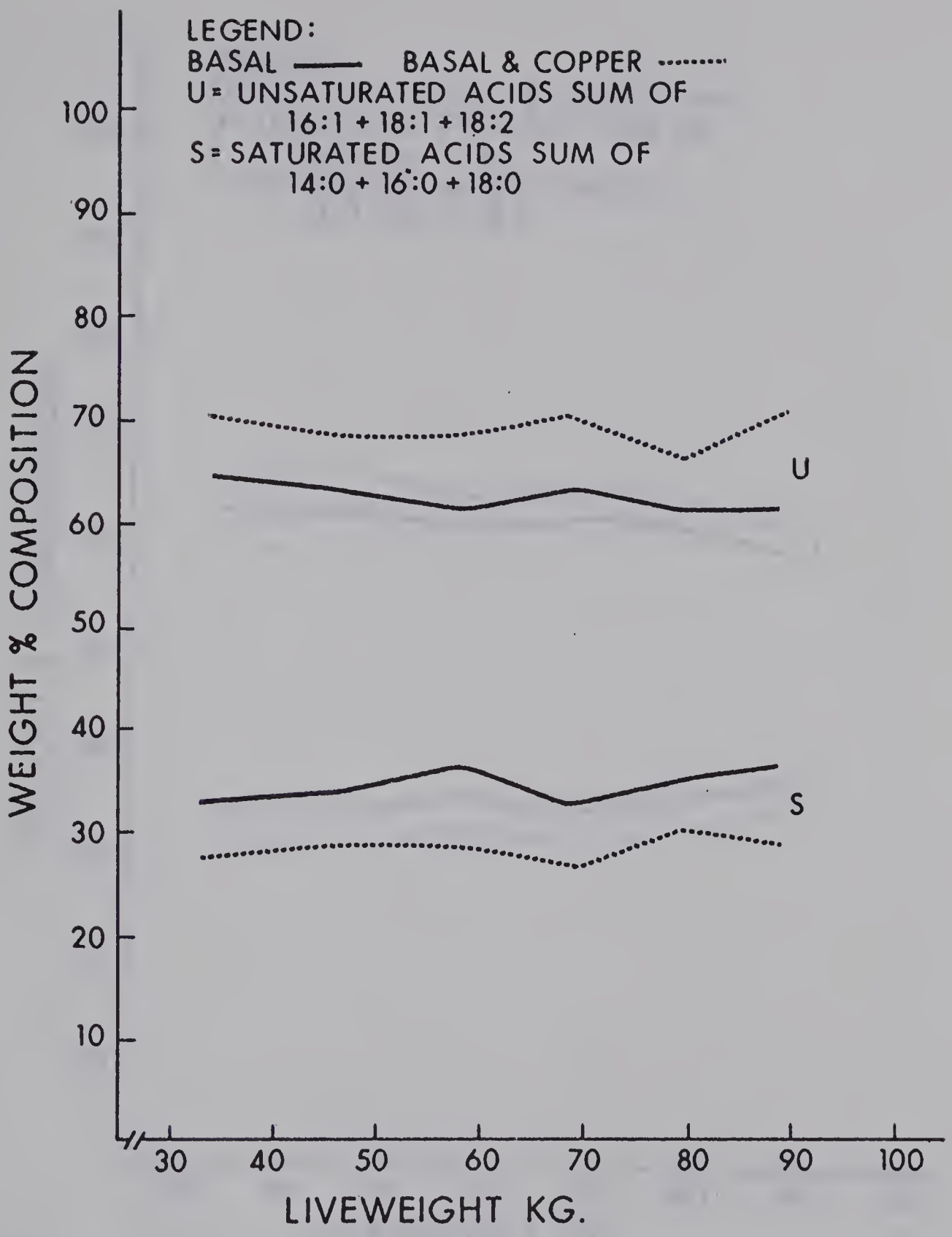


Figure 6. A comparison of levels of saturated and unsaturated fatty acids present in the backfat of pigs fed barley-meat meal diets, with or without supplemental copper, ad libitum.

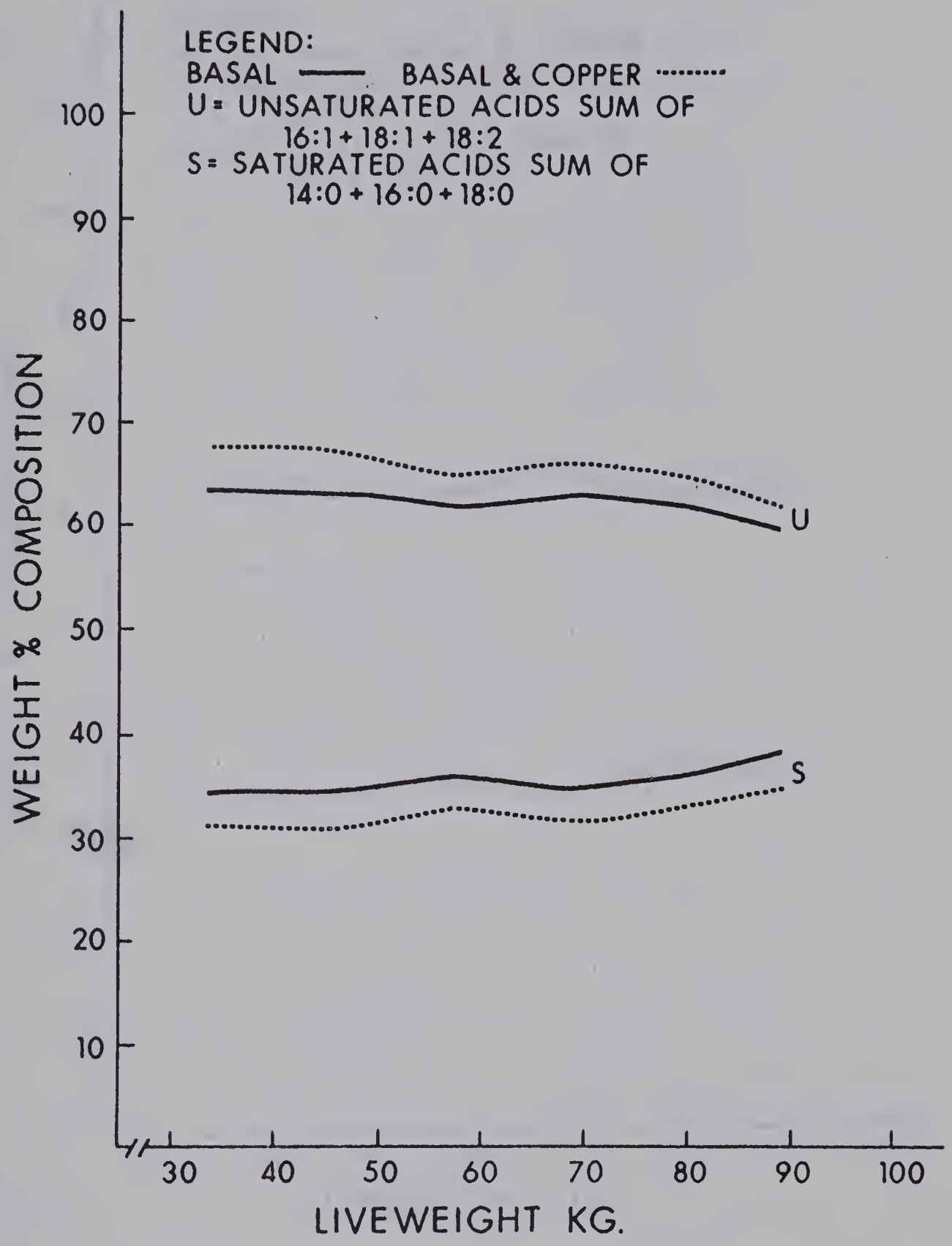


Figure 7. A comparison of levels of saturated and unsaturated fatty acids present in the backfat of pigs fed barley-soybean meal diets, with or without supplemental copper, ad libitum.

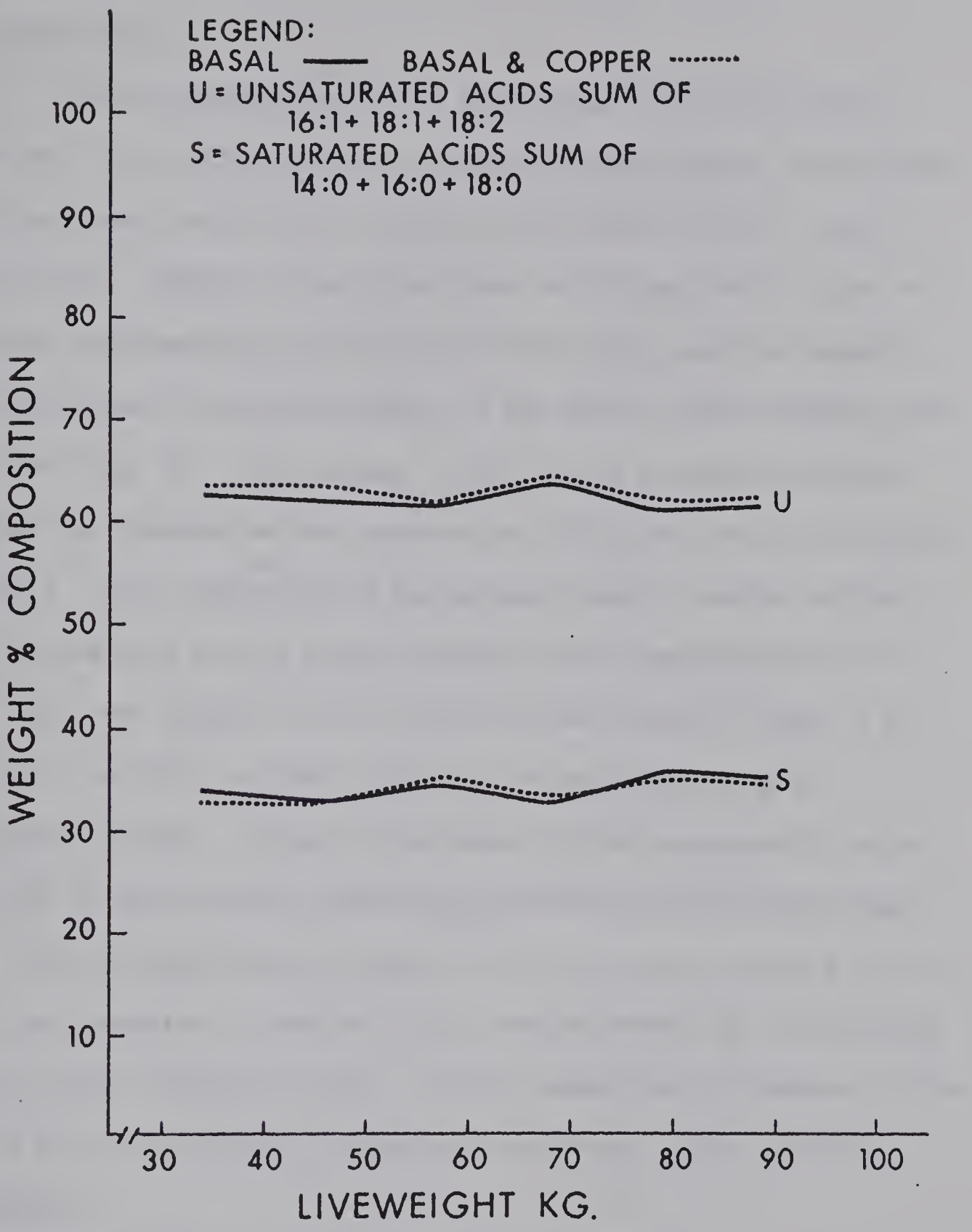


Figure 8. A comparison of levels of saturated and unsaturated fatty acids present in the backfat of pigs fed barley-rapeseed meal diets, with or without supplemental copper, ad libitum.

supplemented pigs.

Copper supplementation of barley-meat meal and barley-soybean meal diets also resulted in significantly higher proportions of UFA and lower proportions of SFA at all weights (Figs. 6 and 7, respectively). However, the proportions of UFA and SFA in pigs fed the copper supplemented barley-rapeseed meal diet were not significantly different from those present in the OBF of pigs receiving the basal diet (Fig. 8). The changes in fatty acid composition which accounted for changes in the proportions of UFA and SFA are presented in Table 8. Pigs receiving the barley-meat meal or barley-soybean meal diets as well as the barley-fishmeal diet supplemented with copper had lower weight % of 16:0 and 18:0 and greater weight % of 16:1, 18:1, and 18:2 in their OBF than did pigs receiving the unsupplemented diets. These differences did not consistently occur in the OBF of pigs fed the copper supplemented barley-rapeseed meal diets. These changes agree in part with the results of Moore et al. (1968), who reported increases in 18:1 and decreases in 18:0 with no change in other component acids. In the present work, changes in the weight % of 16:0, 16:1 and 18:2 also contributed to the change in composition.

The greatest response to copper supplementation occurred in the OBF of pigs receiving the barley-fishmeal diet followed by the barley-meat meal, barley-soybean meal and barley-rapeseed meal diets in that order (Table 9). This observation indicates a greater response to copper supplementation in diets containing animal protein supplements as compared to diets containing vegetable protein

TABLE 8

PERCENT FATTY ACID COMPOSITION OF THE OUTER BACKFAT OF PIGS FED BARLEY DIETS SUPPLEMENTED WITH FISHMEAL, MEAT MEAL, SOYBEAN MEAL OR RAPESEED MEAL WITH OR WITHOUT SUPPLEMENTAL COPPER AS DETERMINED AT SEVERAL LIVEWEIGHTS

Protein Supplement	Liveweight kg	34		45		57		68		79		88		overall	
	Supplemental copper	0	+	0	+	0	+	0	+	0	+	0	+	0	+
	Fatty acid														
Fish-meal	14:0	1.6	2.0[a]	1.3	1.7[a]	1.5	2.0[b]	1.5	1.8[b]	1.7	1.7[b]	1.3	1.5[a]	1.5	1.8[a]
	16:0	19.0	17.3[b]	20.3	17.2[b]	20.8	16.5[b]	20.7	16.0[b]	23.9	18.6[b]	22.5	19.1[b]	21.2	17.4[b]
	16:1	5.6	7.9[a]	5.0	8.2[a]	4.8	8.2[a]	4.3	7.9[a]	4.1	7.6[a]	3.5	6.4[a]	4.5	7.7[a]
	18:0	8.0	5.4[b]	8.4	6.1[b]	10.5	6.0[b]	8.9	5.5[b]	8.9	7.1	11.7	7.7[b]	9.4	6.3[b]
	18:1	51.2	51.2	50.0	50.1	50.2	52.5[a]	51.5	52.0[a]	51.2	52.0[a]	49.4	52.2	50.6	51.7[a]
	18:2	12.2	14.1[a]	11.8	13.7	9.4	12.4[a]	10.7	14.2[a]	8.1	10.4[a]	8.7	10.0	10.1	12.4[a]
	18:3 + 20:0	2.4	2.2	3.2	3.0	2.9	2.4	2.3	2.5	1.8	2.1	2.1	2.3	2.4	2.4
Meat meal	14:0	1.5	1.5[b]	1.4	1.6[b]	1.6	1.6[b]	1.7	1.6[b]	1.4	1.6	1.1	1.4[a]	1.4	1.6
	16:0	21.2	18.8[b]	22.4	19.1[b]	23.0	19.5[b]	21.4	19.0[b]	22.6	21.1[a]	23.5	19.5[b]	22.3	19.5[b]
	16:1	4.0	6.3[a]	3.8	6.3[a]	3.3	6.0[a]	3.3	6.3[a]	2.9	5.4[a]	2.6	4.5[a]	3.3	5.8[a]
	18:0	10.2	7.1[b]	10.1	7.4[b]	11.2	7.5[b]	10.0	6.6[b]	11.2	7.4[b]	11.9	8.1[b]	10.8	7.4[b]
	18:1	50.0	51.3	50.4	50.6	49.8	52.6	50.8	51.8	51.2	52.5	51.1	54.1	50.5	52.2[a]
	18:2	10.5	12.4[a]	9.5	11.6[a]	8.7	10.3[a]	10.3	11.9	7.8	9.2	7.6	9.5[a]	9.1	10.8[a]
	18:3 + 20:0	2.5	2.6	2.6	3.3	2.5	2.5	2.4	2.9	2.4	2.6	1.6	2.2[a]	2.4	2.7

..... cont'd

TABLE 8. continued

Protein Supplement	Liveweight kg	34		45		57		68		79		88		overall	
	Supplemental copper	0	+	0	+	0	+	0	+	0	+	0	+	0	+
	Fatty acid														
Soybean meal	14:0	1.5	1.5	1.5	1.3	1.6	1.4	1.4	1.5$_b$	1.5	1.6$_b$	1.5	1.5$_b$	1.5	1.5$_b$
	16:0	22.1	20.2	22.7	20.9	22.9	21.8	22.3	20.5^b	23.8	22.4^b	24.3	22.7^b	23.0	21.4^b
	16:1	3.9	5.0	4.0	4.8$_b$	3.9	4.7	3.5	5.1	3.5	4.4^a	3.1	3.8	3.6	4.6^a
	18:0	10.5	8.9	10.3	8.2^b	11.2	9.6$_a$	11.1	9.0	10.6	9.1^b	13.0	11.1	11.1	9.3^b
	18:1	46.2	47.6	47.2	47.9	47.3	49.3^a	47.5	48.9	48.3	50.4^a	46.0	47.4	47.1	48.6^a
	18:2	13.2	14.6	11.9	14.2	10.4	10.8	11.9	11.8	9.5	9.9	9.5	10.3	11.1	11.9^a
	18:3 + 20:0	2.7	2.1	2.5	2.8	2.6	2.4	2.2	3.0	2.3	2.0	1.8	2.1	2.4	2.4
Rapeseed meal	14:0	1.6	1.4	1.3	1.3	1.4	1.3	1.4	1.4	1.5	1.6	1.2	1.2	1.4	1.4
	16:0	21.3	20.7	22.5	22.6	22.3	22.8	21.7	22.7	23.0	23.3	22.7	22.1$_a$	22.3	22.3
	16:1	3.5	3.8	3.5	3.5	3.4	3.3	3.2	3.3	3.2	3.0	2.7	3.3^a	3.3	3.3$_b$
	18:0	10.7	10.2	10.2	8.8	10.6	10.5	9.3	8.8	10.8	9.8	11.0	10.5	10.4	9.8^b
	18:1	44.4	45.7	45.6	47.3	46.9	47.5	48.0	48.5	48.2	49.1	48.5	48.2	46.9	47.7
	18:2	14.4	13.7	12.6	12.8	10.9	11.0	12.2	11.2	9.5	9.8	10.0	10.5	11.6	11.5
	18:3 + 20:0	4.0	4.5	4.2	3.9	4.4	3.6	3.9	4.0	3.1	3.0	3.2	3.2	3.8	3.7

Superscript "a" indicates a significantly ($P < .01$ or $< .05$) greater amount of the acid in question in copper supplemented pigs similarly superscript "b" indicates a significantly ($P < .01$ or $< .05$) lesser amount. Absence of a superscript indicates no significant difference.

TABLE 9

RELATIVE AVERAGE CHANGES IN PROPORTIONS OF UNSATURATED AND SATURATED FATTY ACIDS ATTRIBUTABLE TO COPPER SUPPLEMENTATION AS AFFECTED BY SOURCE OF PROTEIN SUPPLEMENT IN THE OUTER BACKFAT OF PIGS.

Source of supplemental protein	Relative change % UFA	SFA
Fishmeal	+6.6	-6.6
Meat meal	+4.1	-4.6
Soybean meal	+3.4	-3.4
Rapeseed meal	+0.8	-0.6

supplements. This is in general agreement with the results of other workers (Barber et al., 1962 and Castell and Bowland, 1968a) who have noted a greater positive effect of copper supplementation on ADG and FC in diets containing animal protein (fishmeal) as compared to vegetable protein (soybean meal).

The decreased response in diets containing soybean meal could be due to the binding of copper by soybean protein (Davis, Norris and Kratzer, 1962) if one assumes that the site of action of copper in fat metabolism is systemic rather than enteric; i.e. that copper must be absorbed and exerts its action at the tissue level. In support of this hypothesis, liver copper values from pigs fed the same barley-fishmeal and barley-soybean meal diets outlined in Table 5 are available (Drouliscos, 1968). Pigs fed the barley-fishmeal diet supplemented with copper had an average liver-copper concentration of

329 ppm (weight/weight) while the average liver-copper concentration for pigs fed the barley-soybean meal diet supplemented with copper was 166 ppm (weight/weight). Possibly the protein of rapeseed meal may have a similar, but more extensive, complexing action on copper than does the soybean protein thus accounting for the lack of response to copper supplementation in pigs receiving the barley-rapeseed meal diet.

In order to compare the results of Experiment 2 for the barley-fishmeal diet, with or without supplemental copper, with the results of Experiment 1, one must assume that the difference in the crude protein level of the barley-fishmeal diets used in the two experiments, 17.7 and 13.8% for Experiments 1 and 2 respectively, would not significantly alter the effect of dietary copper on fatty acid composition. It was noted in Experiment 1 that the effect of copper on fat composition may be related to feeding system or to age. In previous experiments (Bowland and Castell, 1964, 1965) a high incidence of soft fat was noted in the carcasses of pigs fed copper supplemented diets ad libitum when they reached market at 155 days of age. In Experiment 1 in which pigs were fed to a restricted scale based on liveweight (Table 2) the proportion of UFA and SFA in the OBF of copper supplemented pigs at a market weight of 88 kg and an age of 202 days was not significantly different from that of pigs receiving the basal diet. In the present experiment in which the pigs were fed ad libitum, those receiving the barley-fishmeal diet averaged 177 days of age at market. Their OBF at this time was significantly softer having higher levels of UFA and lower levels

of SFA than the OBF of pigs receiving the unsupplemented diet (Fig. 5).

The levels of UFA and SFA present in the OBF in this experiment were significantly affected by weight (or age) of the animal (Table 7). As liveweight increased the proportions of UFA decreased and the proportions of SFA increased resulting in a more saturated fat (Table 7). This observation is in agreement with the finding of Elson et al. (1963), Allen and Bray (1964), Sink et al. (1964) and Allen et al. (1967). If this increase in saturation were related more to chronological age than to weight, then one might hypothesize a balance between two opposing factors. Firstly, there was the tendency of copper supplementation to increase the proportions of UFA and decrease the proportions of SFA in the OBF. Secondly, there was the opposing tendency of the OBF to contain higher proportions of SFA and lower proportions of UFA with increasing age of the animal. A point should eventually be reached whereby these two trends cancel each other resulting in no difference between animals fed copper-supplemented diets and those fed unsupplemented diets. According to the results of Experiment 1, using a restricted feeding system, this point was reached prior to market weight (approximately 90 kg liveweight) when the animals were approximately 200 days of age. However, when ad libitum feeding was practiced and the animals were younger (approximately 177 days of age) at market weight, the tendency for copper supplementation to soften the depot fat was still stronger than the natural tendency of the depot fat to become more saturated. The end result of this was that under conditions of ad libitum feeding, copper supplementation of the diet resulted in carcasses with

soft fat. There is, however, some conflicting evidence in the literature. It has been reported that restricted feeding produces softer fat than ad libitum feeding (Ellis and Zeller, 1931; Shorrock, 1940; Babatunde et al.,1967; Greer et al.,1965).

There were significant two-factor interactions of copper with protein, sex and weight which affected the proportions of UFA and SFA. Means for these interactions are given in Table 7, while the analysis of variance is presented in Appendix Table 1.

The copper x protein interaction which has already been discussed resulted from the greater response to copper supplementation in diets containing animal protein as compared to diets containing vegetable protein. This interaction is plotted in Fig. 9. The copper x weight interaction resulted from a marked decrease in response to copper supplementation at 79 kg (Fig. 9). The copper x sex interaction (Fig. 9) resulted from a greater increase in the proportion of UFA and decrease in the proportion of SFA in copper-supplemented gilts than in copper-supplemented barrows. No explanation can be given for these interactions.

In Experiment 2, the change in proportions of UFA in response to copper supplementation of the barley-fishmeal, barley-meat meal and barley-soybean meal diets could be accounted for in part by significant increases in the weight % of 18:2 present in the OBF (Table 8). This was not a consistent observation in Experiment 1, (Table 3). Linoleic acid is an essential fatty acid generally considered not to be synthesized by pigs and therefore required in the diet. Increases in the proportions of 18:2 in the OBF would

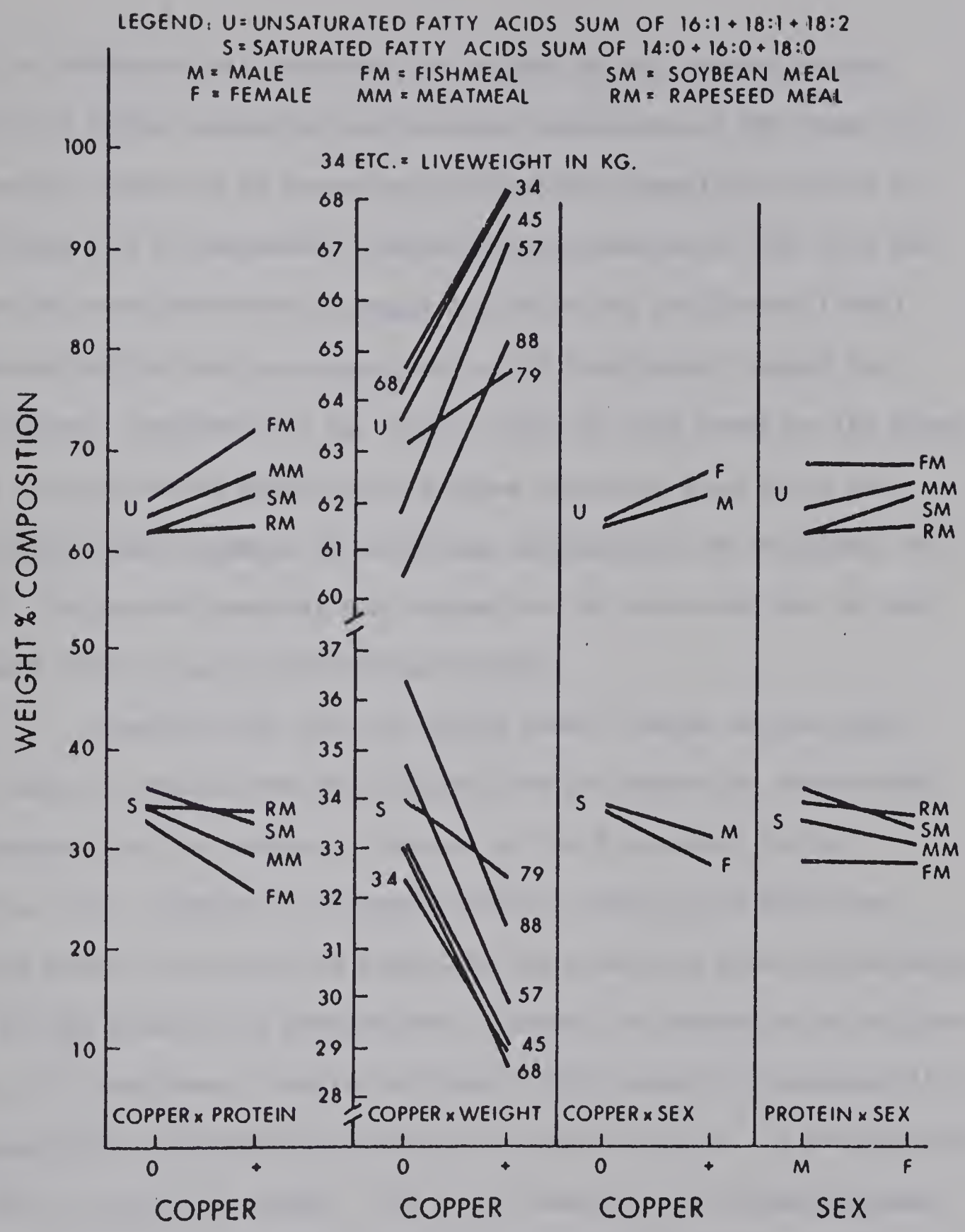
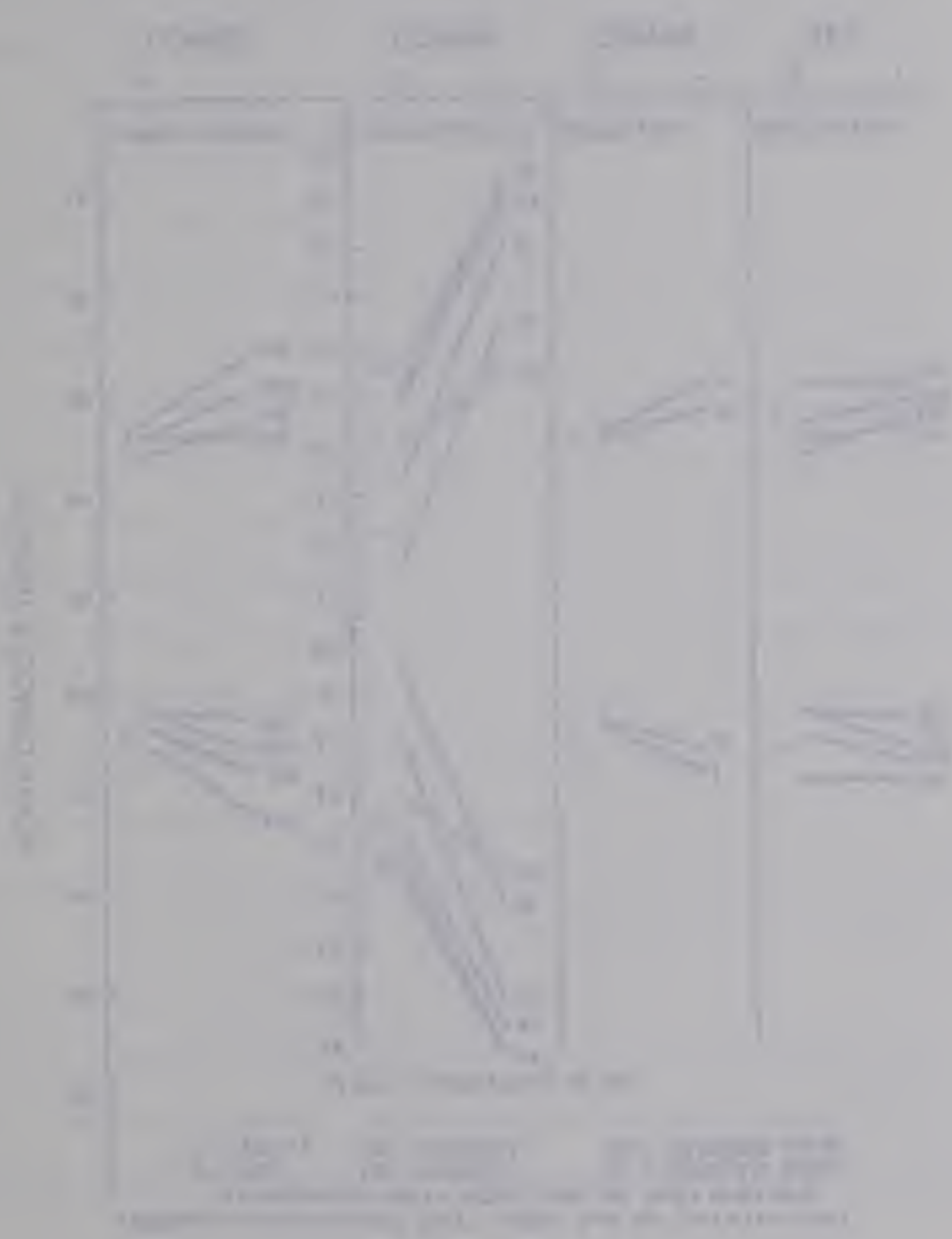


Figure 9. Two factor interactions affecting the proportions of saturated and unsaturated fatty acids in the backfat of pigs.



tend to indicate that the effect of copper on fat metabolism may be one of either promoting preferential absorption of UFA from the intestinal tract or of promoting preferential deposition of UFA in the depot fat or conversely preventing mobilization of UFA from the depot fat once deposited as suggested by Taylor and Thomke (1964). The possibility that some synthesis of 18:2 may occur cannot be eliminated. Babatunde et al. (1968) reported that based on the final 18:2 content of the entire body adipose tissue of pigs fed a fat-free diet there appeared to have been considerable net synthesis of 18:2. Fatty acid analysis was carried out in this work and in the present work by gas-liquid chromatography.

Linoleic acid has its double bonds located at the ninth and twelfth carbons from the carboxyl-end and cannot be synthesized by animals for the reasons discussed in the Literature Review Section C-4. However, a di-unsaturated 18 carbon acid with its double bonds located at the sixth and ninth carbons from the carboxyl-end of the molecule is synthesized by animals by desaturation of 18:0 (Fig. 1). Holloway, Peluffo and Wakil (1963) using C^{14} labelled 18:1 demonstrated the synthesis of this acid (18:2 Δ 6, 9)[1] by desaturation of 18:1 in rat liver cells. The acid, however, was chromatogrammed by gas-liquid chromatography in the same general position as 18:2 Δ 9, 12 appearing only as a shoulder on this peak. Resolution of the peak and identification of its components showed that the radio-activity was associated with the 18:2 Δ 6, 9 acid which is synthesized by animals and not with the 18:2 Δ 9, 12 not synthesized by animals.

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[1] (No. of carbon atoms: No. of double bonds, Δ position of double bonds in molecule)

Possibly then, based on the foregoing discussion, the increase in 18:2 noted in the present experiment was the result of an effect of copper on the metabolic pathways concerned in fatty acid synthesis and stimulated not only the increased synthesis of 16:1 and 18:1 but also the synthesis of 18:2 Δ 6, 9. The acid in our chromatograms was identified as 18:2 Δ 9, 12 using a pure standard and comparing retention times. However, it is possible that 18:2 Δ 6, 9 could have been a non-separable component of this peak. Positive proof of this hypothesis awaits further study, however, proof would lead, in conjunction with studies on the synthesis of 16:1 and 18:1 to the conclusion that the site of action of copper in fat metabolism in systemic rather than enteric. Copper has not yet been implicated as essential to the pathways of de novo fatty acid synthesis.

The weight % of individual fatty acids were significantly affected by weight, protein and sex, as well as copper and were also influenced by several interactions. It is not proposed to discuss these effects; however, means and the analysis of variance are presented in Appendix Tables 2 and 3, respectively.

3. Effect of sex

Barrows had an OBF which contained lesser ($P < .01$) amounts of UFA and greater amounts of SFA than did the gilts. These results support similar findings by Friend and Cunningham (1967). A significant protein x sex interaction was found and is illustrated in Fig. 9. This interaction resulted from the fact that the OBF gilts fed the barley-meat meal and barley-soybean meal diets

contained a higher proportion of UFA and a lower proportion of SFA than did similarly fed barrows, while the OBF gilts receiving the barley-fishmeal and barley-rapeseed meal diets contained approximately the same proportions of UFA and SFA as did that of the barrows. No explanation of this interaction is apparent. It cannot be explained by animal versus vegetable protein as the dietary source because in one case gilts fed a diet supplemented with an animal protein (meat meal) or a vegetable protein (soybean meal) displayed a fat composition different from barrows while in the second case (fishmeal and rapeseed meal) they displayed the same fat composition.

(b) Inner backfat and perinephric fat

Samples of the IBF and PF were obtained at slaughter (88 kg) and their fatty acid composition determined. Copper supplementation of barley-fishmeal, barley-soybean meal and barley-meat meal diets increased the proportions of UFA and decreased the proportions of SFA in the IBF but the IBF of pigs fed the barley-rapeseed meal diet was not similarly altered. The changes followed the same trend as did the changes in the OBF in that weight % increases in 16:1, 18:1 and 18:2 were responsible for the increases in UFA and weight % decreases in 16:0 and 18:0 were responsible for the decreases in SFA (Table 10).

In the case of the PF, the increase in proportions of UFA and decrease in the proportions of SFA were significant for copper supplementation of the barley-fishmeal and barley-meat meal diets but not for the barley-soybean meal and barley-rapeseed meal diets (Table 10). The results for the IBF and PF for copper supplementation of the barley-fishmeal diet are in agreement with the results of

TABLE 10

FATTY ACID COMPOSITION AT 88 KG LIVEWEIGHT OF THE INNER BACKFAT AND PERINEPHRIC FAT FROM PIGS FED BARLEY RATIONS SUPPLEMENTED WITH FOUR DIFFERENT PROTEIN SOURCES WITH OR WITHOUT SUPPLEMENTAL COPPER

Protein Supplement		Fishmeal		Meat meal		Soybean meal		Rapeseed meal		Average	
Copper		0	+	0	+	0	+	0	+	0	+
Inner Backfat	14:0	1.4	1.4	1.2	1.2	2.5	1.2	1.2	1.1	1.6	1.2
	16:0	24.8	19.8[b]	24.6	21.3[b]	24.6	23.7	24.0	24.8	24.5	22.4[b]
	16:1	2.5	5.4[a]	2.4	4.2[a]	2.5	2.8	2.4	2.6	2.4	3.7[a]
	18:0	14.2	10.2[b]	14.7	10.4[b]	15.3	13.6[b]	14.4	13.1	14.6	11.8[b]
	18:1	47.2	50.1	48.5	51.7[a]	44.2	46.5[a]	45.3	46.7	46.3	48.7[a]
	18:2	7.5	10.2[a]	6.5	7.3	8.2	9.4[a]	9.5	8.4	8.0	8.8[b]
	18:3 + 20:0	1.8	2.2	1.4	3.0	2.0	2.2	3.0	2.8	2.0	2.6[a]
Perinephric Fat	14:0	1.5	1.5	1.3	1.4[b]	1.6	1.5	1.4	1.4	1.4	1.4[b]
	16:0	26.0	20.8[b]	26.6	23.5[b]	26.1	27.0	26.7	27.4	26.4	24.6[b]
	16:1	3.2	4.5[a]	2.2	3.6[a]	2.8	2.5	2.2	2.5	2.6	3.3[a]
	18:0	16.1	11.3[b]	17.4	12.5[b]	17.4	16.7	15.6	15.2	16.6	14.0[b]
	18:1	43.0	48.0[a]	44.4	47.8	40.0	41.4	39.9	41.6	41.8	44.7[a]
	18:2	8.0	10.5[a]	6.4	8.2[a]	9.4	9.3[b]	10.7	8.8	8.6	9.2
	18:3 + 20:0	1.7	2.5[a]	1.2	1.9[a]	2.2	1.0[b]	2.8	2.5	2.0	2.0

Superscript "a" indicates a significantly ($P < .05$) greater amount of the fatty acid in question in copper supplemented pigs while superscript "b" indicates a significantly ($P < .05$) lesser amount. Absence of a superscript indicates no significant difference.

Experiment 1 in that the trends observed in the OBF were also observed in the IBF and PF. Means for the effects of protein sources, copper, sex and interactions among these factors on the fatty acid composition of the IBF and PF are presented in Appendix Tables 4 and 5, respectively and the analysis of variance in Appendix Table 6. The general trends are similar to those obtained for OBF at market weight.

The overall effect of copper supplementation on the proportions of SFA and UFA present in the OBF, IBF and PF is presented in Fig. 10. Copper supplementation of the diet resulted in a decrease in the proportion of SFA and increase in the proportion of UFA in these depot fats. In addition, Fig. 10 illustrates the trend to increasing saturation of the depot fat as one moves from outer areas to internal areas which is in agreement with the observations of others (Hilditch and Williams, 1964; Sink et al.,1964; Stinson et al.,1967).

D. SUMMARY

Sixty-four Hampshire x Yorkshire pigs were fed either a barley-fishmeal, barley-meat meal, barley-soybean meal or a barley-rapeseed meal diet with or without 250 ppm supplemental copper. Copper supplementation of the diets did not affect the growth or carcass characteristics of the pigs, however, source of supplemental protein and sex did.

Copper supplementation of the diets increased the weight % of 16:1, 18:1 and 18:2 in the OBF, IBF and PF of pigs fed the barley-fishmeal, barley-meat meal and barley-soybean meal diets but not of pigs fed the barley-rapeseed meal diet. Increases in 16:1, 18:1 and 18:2 were accompanied by decreases in 16:0 and 18:0.

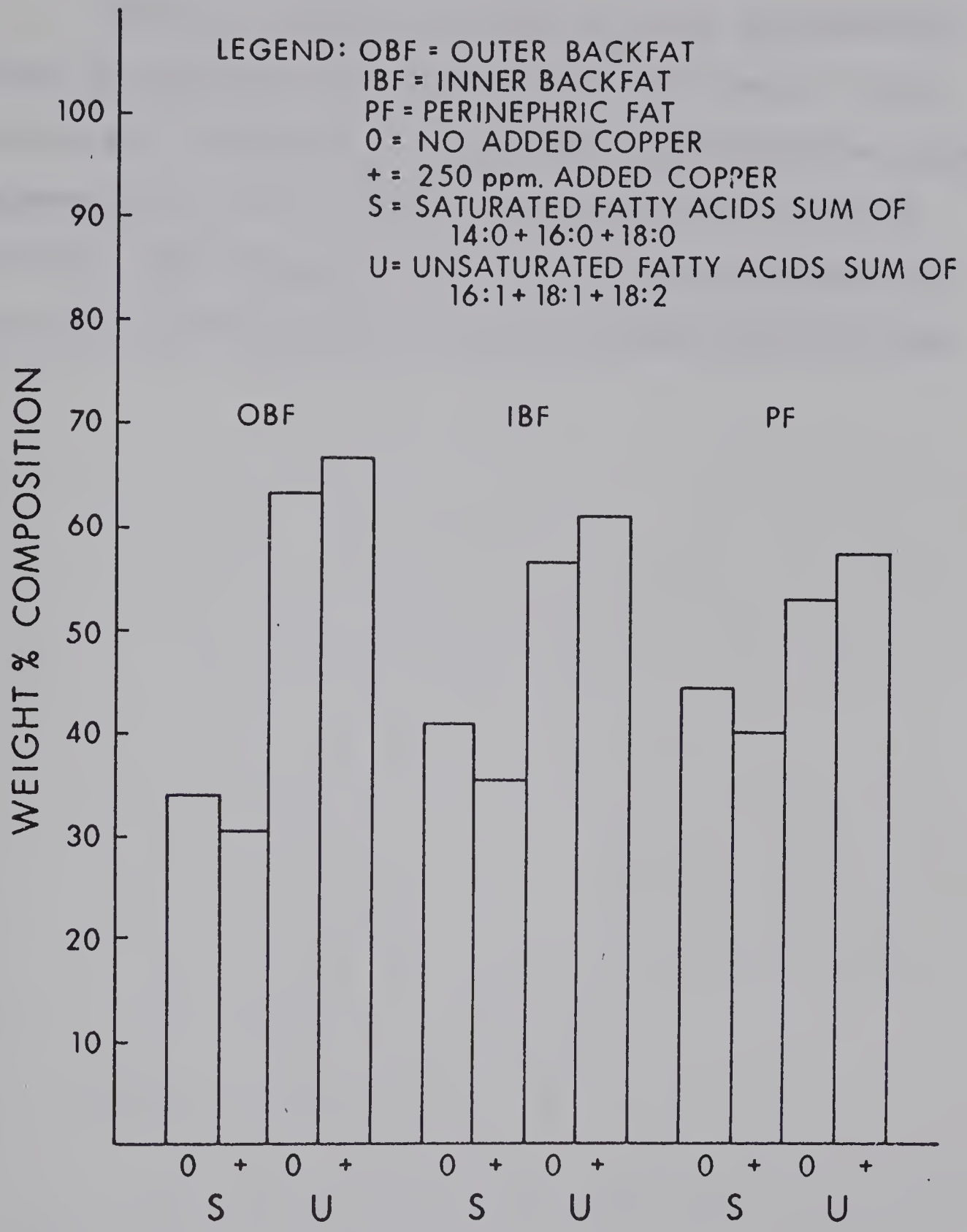

Figure 10. A comparison of levels of saturated and unsaturated fatty acids, in the outer backfat, inner backfat and perinephric fat of pigs fed diets with or without supplemental copper.

Hypotheses regarding the effect of copper supplementation on depot fat composition have been proposed. The results of this experiment and of Experiment 1 support other observations that copper supplementation of the diet exerts a softening effect on porcine depot fats. This softening of depot fats by copper supplementation of the diet is also dependent on source of dietary protein and sex.

GENERAL DISCUSSION

1. Mode of action

The data provide no definite insight into the site or mode of action of copper in fat metabolism. The level of crude fat present in the diets fed is considered too low to significantly affect the composition of the depot fat even if copper, as suggested by Taylor and Thomke (1964), exerted an effect on fatty acid absorption from the intestinal tract combined with an effect on the dynamic state of fatty acids in the body. The observed changes could result from the alteration of known metabolic pathways for the endogenous synthesis of fatty acids. The apparent increase in 18:2 resulting from dietary copper in the second experiment may be, in fact, an increase in the 6, 9 unsaturated 18 carbon acid which animals can synthesize rather than an increase in the 9, 12 unsaturated 18 carbon acid considered essential. Based on these hypotheses the site of action of high level dietary copper on fat metabolism may be associated with the pathways of de novo synthesis of fatty acids.

2. Practical implications

The research reported herein has many practical implications, both advantageous and disadvantageous. Most research designed to evaluate copper as a growth promotant, has indicated that copper supplementation of the diet improves ADG and FC and results in pigs going to market at a younger age and at lower cost per unit of gain. Although, the present work did not confirm this observation, improved ADG and FC could result in considerable saving to the producer of pigs.

Copper added to swine diets at a level of 250 ppm, or at any level greater than 25 ppm, is considered to be a medicating drug (Purdy, 1968). As such, approval from the Food and Drug Directorate must be obtained before copper supplementation of diets can be practiced by feed manufacturers in Canada.

On December 30, 1968, the Canada Department of Agriculture implemented a new hog carcass valuation system. Under this new system, hog carcasses are given an index based on carcass weight and total backfat thickness. The bid price is on an index of 100 and carcasses with an index greater or less than 100 are valued at the bid price multiplied by the index. A quality demerit of 10 points is applied for a soft oily carcass and this is subtracted from the carcass index before multiplying by the bid price. For example:

Carcass weight	150 lb.
Total backfat	3.2 inches
Index	100
Bid price	$30.00/100 lb.
Value of carcass	$30.00 x 1.5 = $45.00
Quality demerit	(soft, oily carcass) - 10 points
New index	100 - 10 = 90
New value	$30.00 x .9 x 1.50 = $40.50
Difference	$45.00 - 40.50 = $4.50

From the foregoing example, a quality demerit of 10 points for a soft oily carcass could cost a producer $4.50. If the carcass had an index of 105 and thereby qualified for the government bonus of $3.00, a quality demerit of 10 points would result in an additional

loss. Therefore, copper supplementation of practical swine diets, would not, on the basis of the data presented herein, appear to be economically advantageous owing to the danger of copper supplementation of the diet resulting in a softer, oilier depot fat in a percentage of the pigs.

Lipid composition may be an important factor in meat quality and in the keeping characteristics of meat. Fats containing relatively high proportions of unsaturated fatty acids are subject to oxidation, the end products of which result in undesirable odours and flavours in the product with which the fat is associated. Therefore, any treatment, such as copper supplementation of practical swine diets, which increases the proportion of unsaturated fatty acids present in the depot fat, could result in adverse effects on the storage life of pork.

Recently, Koch et al. (1968b), have suggested that since the composition of porcine depot fats can easily be altered by dietary means, they could serve as a potential source of unsaturated fat in the human diet for the reduction of serum cholesterol levels. The implications of such reasoning are far-reaching. Based on the results presented herein copper supplementation of pig diets could offer an alternative to the use of highly unsaturated fats in the diet as a means of producing a more unsaturated fat for the human diet.

Further research is required before the practical effects of copper supplementation of swine diets on the fatty acid composition of porcine depot fat can be fully elucidated.

GENERAL SUMMARY

Studies were conducted to determine the effects of supplementing swine diets with copper at a level of approximately 250 ppm, on the fatty acid composition of porcine depot fat. The studies were initiated following the observation at this institution that pigs fed diets supplemented with copper displayed soft fat and a report indicating that copper supplementation of the diet increased the iodine number of porcine depot fats. Barley diets supplemented with fishmeal, meat meal, soybean meal or rapeseed meal were fed with or without supplemental copper.

In general, copper supplementation of barley-fishmeal, barley-meat meal, and barley-soybean meal diets resulted in an increased proportion of UFA and a decreased proportion of SFA in the OBF, IBF and PF. The increase in the proportion of UFA resulted from increases in the weight % of 16:1, 18:1 and 18:2 present in the depot fats. Concomitantly, decreases in the weight % of 16:0 and 18:0 present in the depot fats accounted for the decrease in the proportion of SFA. The effect of copper supplementation of the diet on fatty acid composition was most pronounced among pigs fed the barley-fishmeal and barley-meat meal diets, less pronounced among pigs fed the barley-soybean meal diet and non-existent among pigs fed the barley-rapeseed meal diet.

Feeding system (restricted versus ad libitum) and/or age at market weight affect the change in fat composition resulting from copper supplementation as measured at market weight (88 kg). Pigs fed a copper supplemented barley-fishmeal diet to a restricted scale,

and therefore chronologically older at market weight, tended to display a fat composition not significantly different from control animals fed the basal diet. However, when the same diet was fed ad libitum, and the pigs were chronologically younger at market weight, the fat composition of copper supplemented pigs was significantly different from that of pigs receiving the basal diet.

Fatty acid composition of porcine depot fat was also affected by sex, and interactions of weight and sex with level of added copper and source of supplemental protein.

In summation, based on the results of the experiments reported here, the use of copper supplements in swine rations, at least under ad libitum feeding conditions for rations containing fishmeal, soybean meal or meat meal, cannot be recommended.

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APPENDIX

TABLE 1. Analysis of variance of the sum of the saturated and the sum of the unsaturated fatty acids. Experiment 2.

Source of variation	df	Mean squares: Sum of saturated fatty acids	Mean squares: Sum of unsaturated fatty acids
Protein	3	553.15**	754.95**
Sex	1	285.31**	200.97**
Protein x sex	3	41.019**	39.622**
Copper	1	1382.4**	1312.0**
Protein x copper	3	148.88**	139.18**
Sex x copper	1	61.120**	67.334**
Protein x sex x copper	3	96.127**	69.169**
Weight	5	135.05**	135.49**
Protein x weight	15	9.3109	12.415
Sex x weight	5	6.6225	4.4646
Protein x sex x weight	15	8.1637	7.9732
Copper x weight	5	21.751*	22.252*
Protein x copper x weight	15	25.126**	25.645**
Sex x copper x weight	5	5.9004	7.0879
Protein x sex x copper x weight	15	6.3321	4.5691
Error	288	7.5109	7.4407
Total	383		
Pooled, corrected EMS[1]	262	8.6728	8.4406

[1]Sum of squares for error and P x S x C x W were pooled and divided by df value corrected for missing values (288 + 15 - 41) to arrive at a pooled corrected error mean square (EMS) which was used to test for significance.

*Significant ($P < .05$).

**Significant ($P < .01$).

TABLE 2. Mean weight percent fatty acid composition of the outer backfat of pigs as affected by weight, protein source, sex, copper and certain interactions. Experiment 2.

Factor	Fatty acid:	Weight % fatty acid 14:0	16:0	16:1	18:0	18:1	18:2	18:3 + 20:0
Weight, kg	34	1.58	20.08	5.00	8.87	48.47	13.14	2.88
	45	1.42	20.95	4.89	8.68	48.64	12.26	3.19
	57	1.56	21.20	4.70	9.65	49.52	10.48	2.93
	68	1.55	20.51	4.60	8.67	49.90	11.78	2.91
	79	1.57	22.33	4.26	9.39	50.36	9.28	2.41
	88	1.34	22.06	3.75	10.61	49.61	9.53	2.32
Protein source	FM	1.64	19.31	6.13	7.86	51.14	11.30	2.43
	MM	1.50	20.92	4.55	9.08	51.35	9.94	2.51
	SM	1.48	22.21	4.14	10.22	47.84	11.51	2.38
	RM	1.38	22.31	3.30	10.09	47.33	11.56	3.75
Sex	M	1.48	21.90	4.38	9.54	49.44	10.43	2.63
	F	1.52	20.48	4.68	9.08	49.39	11.73	2.91
Copper	0	1.46	22.20	3.69	10.44	48.79	10.48	2.74
	+	1.55	20.18	5.38	8.18	50.04	11.67	2.80
Protein x copper	FM 0	1.50	21.18	4.55	9.42	50.58	10.14	2.44
	+	1.78	17.44	7.72	6.29	51.70	12.45	2.43
	MM 0	1.45	22.33	3.30	10.79	50.54	9.07	2.35
	+	1.56	19.51	5.81	7.36	52.15	10.82	2.68
	SM 0	1.50	23.02	3.64	11.14	47.09	11.09	2.36
	+	1.47	21.40	4.64	9.31	48.58	11.94	2.40
	RM 0	1.39	22.26	3.26	10.43	46.93	11.63	3.81
	+	1.38	22.35	3.34	9.75	47.74	11.49	3.69
Sex x copper	M 0	1.45	22.68	3.77	10.42	48.85	10.04	2.55
	+	1.52	21.11	4.98	8.65	50.02	10.81	2.71
	F 0	1.46	21.71	3.60	10.47	48.72	10.92	2.93
	+	1.58	19.25	5.78	7.70	50.07	12.53	2.89

TABLE 3. Analysis of variance of the weight % fatty acid composition of the outer backfat of pigs. Experiment 2.

Source of variation	df	Fatty acid:	Mean squares 14:0	16:0	16:1	18:0	18:1	18:2	18:3+20:0
Weight	5		0.60398**	48.827**	13.568**	36.021**	34.296**	155.36**	7.2533**
Protein	3		1.0249**	188.31**	135.19**	115.27**	433.56**	56.356**	41.383**
Weight x protein	15		0.11960	3.8032	0.72893	3.3483	9.3695*	2.7532	0.80645
Sex	1		0.12760	192.10**	8.8512*	19.711*	0.20174	163.37**	7.1777*
Weight x sex	5		0.00648	5.6195	0.44715	1.5155	6.7215	6.1138	0.68904
Protein x sex	3		0.27240	26.212**	7.6182**	2.6289	9.4808	6.8461	1.7563
Weight x protein x sex	15		0.15960	1.6377	0.92451	3.4501	5.2162	5.3070*	0.56082
Copper	1		0.73500*	392.04**	272.87**	493.23**	152.01**	135.49**	0.34440
Weight x copper	5		0.01362	0.89454	0.75286	0.88960	3.8735	0.46196	1.1791
Protein x copper	3		0.49257*	65.933**	47.433**	38.267**	3.2313	27.614**	0.87995
Weight x protein x copper	15		0.10186	3.6857	0.50469	1.8608	3.0781	2.3141	0.44231
Sex x copper	1		0.08167	18.904*	22.089**	23.800**	0.75254	17.298*	0.91066
Weight x sex x copper	5		0.05429	0.99175	1.5057	1.2790	1.3087	1.4556	0.60621
Protein x sex x copper	3		0.12590	32.198**	15.066**	24.448**	16.914*	7.2311	1.7776
Weight x protein x sex x copper	15		0.08819	2.5478	1.7356	2.9835	6.3026	3.6152	1.0578
Error	288		0.10373	2.6995	1.0592	2.6169	4.7300	2.4823	0.97356
Total	383								
Pooled, corrected EMS[1]	262		0.11910	3.1132	1.2636	3.0474	5.5601	2.9357	1.1307

[1]Sum of squares for error and P x S x C x W were pooled and divided by a df value corrected for missing values (288 + 15 - 41) to arrive at a pooled, corrected error mean square (EMS) which was used to test for significance.

*Significant (P .05).

**Significant (P .01).

TABLE 4. Mean weight % fatty acid composition of the inner backfat of pigs as affected by protein source, sex, copper and interactions. Experiment 2.

Factor	Fatty acid:	Weight % fatty acid 14:0	16:0	16:1	18:0	18:1	18:2	18:3 + 20:0
Protein source	FM	1.39	22.25	3.94	12.22	48.66	8.89	2.02
	MM	1.17	22.97	3.28	12.57	50.10	6.92	2.22
	SM	1.84	24.15	2.68	14.46	45.33	8.79	2.08
	RM	1.16	24.39	2.47	13.72	45.98	8.98	2.91
Copper	0	1.57	24.50	2.44	14.65	46.29	7.96	2.04
	+	1.21	22.38	3.74	11.83	48.75	8.84	2.57
Sex	M	1.55	23.98	2.91	13.62	47.53	7.43	2.32
	F	1.24	22.90	3.28	12.86	47.50	9.36	2.29
Protein x copper	FM 0	1.38	24.75	2.52	14.24	47.25	7.55	1.84
	+	1.41	19.75	5.36	10.20	50.08	10.24	2.20
	MM 0	1.18	24.64	2.38	14.71	48.48	6.52	1.42
	+	1.16	21.30	4.18	10.42	51.72	7.32	3.01
	SM 0	2.53	24.58	2.51	15.29	44.16	8.24	1.95
	+	1.15	23.72	2.84	13.62	46.50	9.35	2.21
	RM 0	1.20	24.02	2.35	14.35	45.28	9.51	2.96
	+	1.12	24.75	2.59	13.09	46.69	8.45	2.85

TABLE 5. Mean weight % fatty acid composition of the perinephric fat of pigs as affected by protein source, sex, copper and interactions. Experiment 2.

Factor		Fatty acid:	Weight % fatty acid 14:0	16:0	16:1	18:0	18:1	18:2	18:3 + 20:0
Protein source	FM		1.49	23.37	3.88	13.68	45.54	9.25	2.12
	MM		1.36	25.08	2.91	15.14	46.11	7.29	1.54
	SM		1.54	26.56	2.62	17.05	40.67	9.35	1.56
	RM		1.38	27.04	2.36	15.39	40.79	9.79	2.63
Copper	0		1.45	26.36	2.61	16.64	41.82	8.61	1.95
	+		1.44	24.66	3.28	13.99	44.73	9.23	1.98
Sex	M		1.46	26.10	2.86	16.04	43.23	7.69	1.92
	F		1.42	24.92	3.03	14.59	43.32	10.15	2.01
Protein x copper	FM	0	1.52	25.95	3.21	16.09	43.02	7.96	1.69
		+	1.46	20.79	4.55	11.26	48.05	10.54	2.55
	MM	0	1.30	26.65	2.25	17.42	44.36	6.35	1.20
		+	1.41	23.50	3.58	12.85	47.85	8.24	1.89
	SM	0	1.59	26.11	2.75	17.40	39.98	9.39	2.15
		+	1.49	27.00	2.50	16.70	41.36	9.31	0.98
	RM	0	1.38	26.74	2.22	15.64	39.92	10.74	2.76
		+	1.39	27.35	2.50	15.15	41.65	8.84	2.50

TABLE 6. Analysis of variance of the weight percent fatty acid composition of the inner backfat and perinephric fat of pigs. Experiment 2.

Area	Source of variation	df	Mean squares 14:0	16:0	16:1	18:0	18:1	18:2	18:3 + 20:0
Inner backfat	Protein	3	1.6352	16.214**	7.0496**	17.077**	80.640**	15.532**	2.6717*
	Sex	1	1.5314	18.598*	2.1756	9.3025	0.01563	59.483**	0.00100
	Protein x sex	3	2.6493	3.7677	0.82604	4.3467	20.692**	1.5531	1.2358
	Copper	1	2.0664	71.614**	27.040**	126.56**	95.530**	12.514*	4.4100
	Protein x copper	3	1.8877	25.978**	6.2579**	9.8683**	2.4928	9.4676*	2.1742
	Sex x copper	1	1.9252	0.97517	1.5625	2.3256	0.15998	0.43891	1.2100
	Protein x sex x copper	3	0.91974	2.1968	0.02292	4.6273	30.137**	5.8910	3.2175*
	Error	48	1.0814	2.9549	0.56864	2.5763	4.7476	2.0817	0.72729
	Total	63							
	Corrected EMS[1]	41	1.2660	3.4595	0.6657	3.0161	5.5580	2.4371	0.8515
Perine-phric fat	Protein	3	0.12182**	43.846**	7.0364**	30.600**	139.28**	19.685**	4.3039**
	Sex	1	0.01891	22.444*	0.47265	33.495*	0.10565	97.269**	0.13141
	Protein x sex	3	0.16932**	8.0477	0.20932	9.6343	4.7852	13.224**	0.22182
	Copper	1	0.00141	46.410**	7.2227**	112.10**	135.14**	6.1876	0.01266
	Protein x copper	3	0.03516	34.846**	2.5027	22.553*	11.372	16.349**	3.5506
	Sex x copper	1	0.00141	3.2852	0.01891	1.9952	6.2500	0.09765	0.03516
	Protein x sex x copper	3	0.04266	1.4506	1.1256	12.307	9.9738	0.37182	0.15057
	Error	48	0.02141	3.8690	0.34213	5.1847	7.7102	2.8678	0.31724
	Total	63							
	Corrected EMS[1]	41	0.02506	4.5295	0.40050	6.0700	9.0266	3.3476	0.37140

[1]Error df were corrected for missing values and the sum of squares for error divided by the corrected df to arrive at a corrected error mean square (EMS).

*Significant (P .05).

**Significant (P .01).